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DNA POLYMERASES IN PROKARYOTES AND EUKARYOTES :
MODE OF ACTION AND BIOLOGICAL IMPLICATIONS

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DNA polymerases in prokaryotes and eukaryotes: Mode of action and biological implications

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Background

DNA transactions such as replication, repair and recombination require the concerted action of many proteins either alone or in a complex¹. Nevertheless, in all these processes one protein, the DNA polymerase, plays a central role. Its function is to ensure the accurate incorporation of the 4 deoxyribonucleoside triphosphates (dATP, dCTP, dGTP and dTTP) on a mother template according to the Watson-Crick base pairing rules (A to T and G to C).

Since the discovery of the first DNA polymerase about 25 years ago² such enzymes have been found in all prokaryotic and eukaryotic organisms tested^{1,3-15,162,163}. Although they differ in chemical, physical or immunological properties they all share the following important similarities: a) they incorporate a complementary deoxyribonucleoside triphosphate as monophosphate on a template by hydrolyzing the α - β phosphodiester bond in the presence of cofactors (divalent cation and DNA), b) they all need a short complementary piece of nucleic acid (DNA or RNA), called primer, with a free hydroxyl group at the third carbon position of the (deoxy)-ribose to attach the first nucleotide and c) they all cause polymerisation in the 5' to 3' direction of the growing daughter strand. It is the aim of this review to compare DNA polymerases from prokaryotes and eukaryotes and especially to point out similarities and differences. In particular, attempts are made to compare *Escherichia coli* DNA polymerases I, II and III¹ with the different vertebrate DNA polymerases α , β and γ ¹⁶. Basic rules of DNA synthesis have been described for prokaryotic and eukaryotic cells and organisms¹. These include, e.g., semiconservative mode of replication, RNA priming, direction of replication, leading and lagging strand replication, excision repair processes. It seems that this extends to the DNA polymerase level since growing pro- and eu-

karyotic cells possess at least three different DNA polymerases^{1,3-16,162,163}.

Biological functions of the different DNA polymerases have been well studied in prokaryotes because of their genetics (conditional lethal mutants of certain DNA polymerase genes), and the ease of obtaining large cell quantities due to the short generation time of bacteria. On the other hand only indirect in vitro experimental evidence is available so far for the involvement of the mammalian DNA polymerases in replication and/or repair. Such descriptive results will always be tentative until conditional lethal DNA polymerase mutants are found. However, there have been reports of mutants in structural genes of DNA polymerases of lower eukaryotic cells, such as fungus and fruit-fly^{17,18}, indicating a direct involvement of these enzymes in chromosomal DNA replication. The DNA polymerase(s) involved in a particular DNA replication process is/are most likely to act in a complex with other proteins (factors) and enzymes, called the replisome¹⁹. Similar concerted actions can also be postulated for DNA repair²⁰ or DNA recombination²¹. In DNA replication the multipolypeptide complex responsible for the overall DNA polymerization is henceforth called the DNA elongation complex or DNA elongation apparatus. In recent years a number of laboratories have described complexes of

Abbreviations

DNA pol:	DNA polymerase (E.C. 2.7.7.7.)
dATP:	Deoxyadenosintriphosphate
dCTP:	Deoxycytidintriphosphate
dGTP:	Deoxyguanosintriphosphate
dTTP:	Deoxythymidintriphosphate
ss:	Single-stranded
ds:	Double-stranded
SDS:	Sodium dodecyl sulfate
ϕ X174, G4, M13:	Bacteriophages ϕ X174, G4 and M13
RF:	Replicative form of a single-stranded DNA

DNA polymerases and accessory proteins. In prokaryotes these proteins are called DNA polymerase III holoenzyme subunits²², DNA elongation factors²³ and DNA polymerase auxiliary proteins²⁴. In eukaryotes on the other hand, there are only fragmentary data about such complexes. Until recently few attempts have been made to use naturally occurring template/primer systems (e.g. singly RNA primed single-stranded DNAs, mitochondrial DNA containing a D-loop or single-stranded parvoviral DNA with a natural primer) to isolate an in vivo-like DNA elongation apparatus^{25,26}. In vitro model replication systems such as papovavirus²⁷⁻²⁹ or adenovirus³⁰ proved to be effective tools in identifying proteins that interact with DNA polymerase(s). The concerted action of several proteins in connection with polymerases helps to achieve the main goal in replication, namely to act promptly, quickly and accurately.

Although there are differences in DNA replication between prokaryotes and eukaryotes, and sometimes even within a single cell type, it is the objective of this review to find and present similarities of DNA polymerizing enzymes from prokaryotic and eukaryotic organisms. Replicative DNA polymerases and their auxiliary proteins are especially good candidates for comparison since their function in all cells is to produce an accurate copy of the genetic material. The high accuracy of these polymerases and their associated proteins ensures the overall consistency of a species but on the other hand their mistakes, although very limited in number (less than $1:10^9$ – $1:10^{10}$), are one of the sources of evolutionary variability³¹.

After a comparative description of prokaryotic and eukaryotic DNA polymerases I will first focus on their respective biological functions. Then, proteins interacting with replicative DNA polymerases are presented. This is followed by the description of interactions of the DNA elongation apparatus with primers at the leading and lagging strand at the replication fork, the processive translocation and the accuracy of polymerisation. Finally some newer aspects will be summarized. These include proteolytic cleavage and the conservation of DNA polymerases in evolution.

1. Description of prokaryotic and eukaryotic DNA polymerases

a) Isolation: Problems and progress

Soon after the discovery in *Escherichia coli* of an enzymatic activity capable of polymerizing the 4 deoxyribonucleoside triphosphates on a DNA template^{2,32} it became clear that a corresponding DNA polymerizing activity also existed in animal cells^{33,34}. Thus it was assumed that such an enzymatic activity is universal. DNA polymerases were subsequently iden-

tified, isolated and characterized in all cells, tissues and organisms tested. About a dozen years later it became evident that prokaryotes and eukaryotes possess several types of DNA polymerases. In *E. coli*, in addition to the above-mentioned DNA polymerase (henceforth called DNA polymerase I), 2 new enzymes were found, named in order of their discovery DNA polymerase II³⁵⁻³⁷ and DNA polymerase III^{38,39}. Also, in animal cells it became clear, even without having conditional lethal mutants, that there are again at least 3 different DNA polymerizing enzymes¹⁶: First a high-molecular-weight DNA polymerase⁴⁰ (henceforth called DNA polymerase α), followed by the small-molecular-weight DNA polymerase β ^{41,42}, and the DNA polymerase γ ⁴³. Finally data were presented suggesting the existence of a 4th polymerase, called δ ⁴⁴.

It is now established that organisms ranging from bacteria to human cells contain several DNA polymerases. It is not the aim of this review to present all the laborious and often controversial work that has been carried out in this field. After describing some of the most highly purified enzymes, from bacteriophage to mammalian ones, I have chosen mainly the DNA polymerases from *E. coli* and vertebrate cells for a detailed comparison.

In *Escherichia coli* the DNA polymerases I^{32,45,46}, II⁴⁷⁻⁴⁹ and III⁵⁰⁻⁵⁴ were purified to apparent homogeneity (for discussion of multiple bands on SDS-polyacrylamide gels of pure DNA polymerase preparations, see below). Bacteriophages exist that code for and possess their own replicase. Examples are: T7^{55,56}, T4⁵⁷, T5⁵⁸ and SPO1⁵⁹. Other prokaryotic cells from which homogeneous or near homogeneous preparations have been reported are: *Bacillus subtilis* DNA polymerase I⁶⁰, II⁶¹ and III⁶², *Micrococcus luteus*^{63,64} and *Salmonella typhimurium*⁶⁵.

In eukaryotes the most extensive and successful efforts were made using a) unicellular and lower eukaryotes such as *Ustilago maydis*⁶⁶, *Saccharomyces cerevisiae*^{67,68}, *Euglena gracilis*⁶⁹, *Physarum polycephalum*⁷⁰, *Paramecium*⁷¹, *Tetrahymena pyriformis*⁷², *Dictyocoelium discoideum*⁷³; b) insects such as *Drosophila melanogaster* (DNA polymerase α ^{74,75} or β ⁷⁶); echinodermata e.g. sea urchins⁷⁷⁻⁸⁰; d) amphibia e.g. *Xenopus laevis* (DNA polymerase α ⁸¹⁻⁸³, β ⁸⁴ and γ ⁸³); e) birds e.g. chicken embryo (DNA polymerase α ⁸⁵, β ⁸⁶ and γ ^{87,88}); and finally f) mammals e.g. rat, calf or human (DNA polymerase α ⁸⁹⁻⁹⁵, β ^{96,97}, γ ^{98,100,360} and the possible δ ¹⁰¹).

Some difficulties should be mentioned at this stage in order to see the often controversial reports in the right perspective. Among these are a) the low amounts of certain DNA polymerases per cell, and b) the small amount of defined material, especially from mammals, owing to limited cell sources and high costs. c) The existence of multiple DNA polymerase species in

all cells and tissues render isolations cumbersome. d) Further complications arise from the occurrence of multiple peaks of DNA polymerases upon ion exchange- and affinity-chromatography; these are not due to different DNA polymerases but rather to different forms of DNA polymerase-protein complexes, as is known today from extensive work with *Escherichia coli* DNA polymerase III holoenzyme^{22,54,102-105}. e) The majority of replicative DNA polymerases, even when purified to homogeneity as judged by electrophoresis in non-denaturing polyacrylamide gels, are themselves found to be complexes of two and more polypeptides^{75,89-95}. The quantitative and/or qualitative variations of DNA polymerases in different physiological stages of cells and organisms is another explanation for controversial findings. Finally f), in early developing and rapidly differentiating tissues, the DNA polymerase structure may be altered by generalized or genetically programmed proteolysis creating fragments still active in *in vitro* assays (see below).

In contrast to the situation of protein synthesis, where tens of thousands of ribosomes are present per cell, enzymology of DNA replication is hampered by the very small quantities of proteins and by inability to isolate the intact replisome. However, some of these obstacles have now been overcome and progress has been made: 1. Genetic engineering allows the overproduction of replication proteins¹⁰⁴, and *in vitro* construction of an intact and fully active prokaryotic replisome has recently been achieved^{19,106-110}. Furthermore, 2. laborious collection of fast replicating tissues such as fly eggs gives reasonable quantities of DNA polymerases and other replication proteins⁷⁵, 3. antibodies¹¹¹⁻¹¹⁶, and inhibitors¹¹⁷⁻¹²⁰ can distinguish between multiple DNA polymerases. 4. Assays for DNA polymerase auxiliary proteins²⁴ and DNA polymerase subunits^{22,102-104,121} can solve the problem of multiplicity of identified DNA polymerizing enzymes. 5. The catalytic subunits of DNA polymerases can now be identified because a) the *dna E* gene of *Escherichia coli* DNA polymerase III can be cloned¹²², b) all other proteins can be removed from prokaryotic or eukaryotic DNA polymerase complexes^{54,123} and c) the catalytic DNA polymerase activity can be regained after SDS-gel electrophoresis^{124,125}. 6. Defined eukaryotic cells and tissues can be used to estimate the quantitative variation pattern of DNA polymerases to provide information about their functional roles. Examples are: Synchronized cell cultures in various phases of their cell cycle^{126,127}, *in vivo* differentiating or proliferating cells such as neurons and spleen cells, respectively¹²⁸⁻¹³⁰, regenerating liver after partial hepatectomy^{131,132} and phytohemagglutinin-stimulated lymphocytes¹³³⁻¹³⁵. 7. Last but not least, the availability of potent protease inhibitors has improved the reproducibility of isolation procedures

and has made comparison between DNA polymerases more meaningful.

b) Physico-chemical and other properties of highly purified or homogeneous enzymes

Detailed properties of DNA polymerases from all kinds of biological sources have been summarized in books and review articles^{1,3-16,162,163}. Especially in the case of eukaryotic DNA polymerases the authors were forced to use information about preparations which were far from being homogeneous. In table 1 an attempt is made to compare a variety of DNA polymerases from organisms ranging from bacteriophages to mammals with respect to four criteria, namely molecular weight, number of subunits, homogeneity (a DNA polymerase is defined as homogeneous if one stained band is seen on a native polyacrylamide gel and this band corresponds to the enzyme activity) and associated enzyme activities.

1. Molecular weight: The replicative DNA polymerases possess high molecular weight (> 110,000-120,000) subunits in their purest fractions (see later sections for functional roles of DNA polymerases, for heterogeneity and also for the problem of generalized proteolysis). From Echinodermata to mammals a small molecular weight (< 50,000) β polymerase can be found. The β -like polymerase from lower eukaryotes has a molecular weight of more than 70,000 daltons. The subunit structure of DNA polymerase γ is controversial but the purest preparations from birds^{87,88} and from mammals³⁶⁰ are suggestive of a small molecular weight (< 50,000) catalytic subunit. All bacteriophage DNA polymerases have a molecular weight of more than 87,000 and bacteria and mycoplasmas all possess DNA polymerases with a molecular weight of more than 105,000 daltons.

2. Number of subunits: It is evident that well-characterized replicative DNA polymerases from bacteriophage T4 and T7, DNA polymerase III holoenzyme from *Escherichia coli*, and eukaryotic DNA polymerases α exist as multipolypeptide complexes. DNA polymerase γ , the mitochondrial replicase (see next section), appears to exist as a tetramer complex.

3. Homogeneity: Due to considerable efforts in the last few years, DNA polymerases from a variety of lower and higher eukaryotes have been obtained in apparently homogeneous form. Work with prokaryotic DNA polymerases has taught us that this is an absolute prerequisite for performing meaningful *in vitro* experiments.

4. Associated enzyme activities are discussed in the following section.

c) Other enzyme activities associated with the DNA polymerase polypeptide chain

Certain DNA polymerases such as DNA polymerase I from *Escherichia coli* are able to carry out several

Table 1. Properties of prokaryotic and eukaryotic DNA polymerases

Organism		Molecular weight ($\times 10^{-3}$) ^a	Number of subunits ^b	Homogeneity ^c	Associated 3' $\rightarrow$ 5' exonuclease	Other associated activities ^d
Bacteriophages	T7	87 (56) ^e	2 (56, 136)	+ (56)	+ (137)	+ (137)
	T4	114 (138)	1 (138) 5 (24) ^f	+ (138)	+ (138)	+ (138)
	T5	96 (139)	1 (58)	+ (58)	+ (58)	+ (58)
Mycoplasmas		106-140 (140, 141)	1 (140, 141)	+ (141)	- (141)	- (141)
	<i>E. coli</i> DNA pol I ^h	109 (46)	1 (46)	+ (45, 46)	+ (142)	+ (143)
	<i>E. coli</i> DNA pol II	120 (47-49)	1 (47-49)	+ (47-49)	+ (47-49)	- (47-49)
Bacteria	<i>E. coli</i> DNA pol III	140 (52)	3 (54)	+ (52, 54)	+ (53, 54)	+ (53, 54)
	<i>B. subtilis</i> DNA pol I	115 (60)	8 (105, 144) ^f			
	<i>B. subtilis</i> DNA pol II	? (61) ^g	1 (60)	+ (60)	?	?
	<i>B. subtilis</i> DNA pol III	166 (62)	1 (62)	+ (61)	+ (61)	- (61)
				+ (62)	+ (62)	+ (62)
				- (68)	- (68)	- (68)
Lower eukaryotes	<i>S. cerevisiae</i> DNA pol I (A)	70 (68)	2 (68)	- (68)	+ (67, 68)	+ (68)
	<i>S. cerevisiae</i> DNA pol II (B)	70 (68)	2 (68)	- (68)	+ (145, 146)	+ (145, 146)
	<i>U. maydis</i> DNA pol A	50-55 (66), > 125 (124)	2-4 (66)	+ (66)	?	?
	<i>U. maydis</i> DNA pol B	? (66)	?	- (66)	?	?
	<i>E. gracilis</i> DNA pol A	142-150 (69), 185 (69)	1-2 (69)	- (69)	- (69)	- (69)
	<i>E. gracilis</i> DNA pol B	38 (69)	2-4 (69), 6 (69)	- (69)	+ (69)	- (69)
	<i>P. polycephalum</i> DNA pol α -like	98-136 (71, 147), 106 (148)	2 (148)	+ (147)	?	?
	<i>P. polycephalum</i> DNA pol β -like	81 (71, 148)	?	- (148)	?	?
	<i>T. pyriformis</i> DNA pol α -like (I)	80 (72), 130 (149)	?	+ (72)	?	+ (72)
	<i>T. pyriformis</i> DNA pol β -like (II)	70 (149)	?	- (149)	?	?
Insects	<i>D. discoideum</i> DNA pol α -like (A)	127 (73)	?	- (73)	?	?
	<i>D. discoideum</i> DNA pol β -like (B)	70 (150)	?	- (150)	?	?
	<i>D. melanogaster</i> DNA pol α	148 (75, 123)	4 (75, 123)	+ (75)	- (75)	- (75)
	<i>D. melanogaster</i> DNA pol β	? (76)	?	- (76)	?	?
Echinodermata	Sea urchin DNA pol α	150 (151)	2 (151)	- (151)	?	?
	Sea urchin DNA pol β	34 (152)	2 (152)	- (152)	?	?
	Sea urchin DNA pol γ	? (78)	?	- (78)	?	?
Amphibia	<i>X. laevis</i> DNA pol α	76 (83)	?	(83)	?	?
	<i>X. laevis</i> DNA pol β	45 (84)	1 (84)	- (84)	?	?
	<i>X. laevis</i> DNA pol γ	? (83)	?	- (83)	?	?
Birds	Chicken embryo DNA pol α	148 (85)	1 (85)	+ (85)	- (85)	- (85)
	Chicken embryo DNA pol β	40 (86)	1 (86)	+ (86)	?	?
	Chicken embryo DNA pol γ	47 (87, 88)	4 (88)	+ (88)	?	?
Mammals	DNA pol α	47-156 (89-95, 125)	2-8 (89-95, 125)	+ (89-95)	- (95) + (91)	- (95) + (91, 153)
	DNA pol δ	49, 60 (101, 154)	2-4 (101, 154)	+ (101, 154)	+ (101, 154)	+ (44)
	DNA pol β	39-45 (96, 97, 155), 32 (157)	1 (96, 97, 155-157)	+ (96, 97, 155-157)	- (97, 153, 156, 158, 159)	- (153, 156) + (97)
	DNA pol γ	140-180 (99, 100, 124), 47 (360), 60 (160)	4 (360)	+ 360	?	?

^a Values taken from SDS-polyacrylamide gels, where possible, or from other molecular weight analyzing techniques, if they were performed under high salt conditions to prevent aggregation. This should give a tentative idea about the molecular weights of the catalyzing subunits. ^b Number of stained bands after SDS-polyacrylamide gel electrophoresis, or oligomeric form of the putative catalytic subunits. ^c Homogeneity means 1. one stained band after SDS-polyacrylamide gel electrophoresis, 2. a single band on a native polyacrylamide gel which subsequently may produce several bands in a SDS-polyacrylamide gel on re-electrophoresis or 3. several bands copurified after extensive purification over several affinity chromatography of gel filtration columns. ^d Examples are: pyrophosphate exchange, pyrophosphorylation, 5' $\rightarrow$ 3' exonuclease activity, RNase H activity. ^e Reference number in brackets, see literature. ^f Including all DNA auxiliary proteins or DNA polymerase III holoenzyme subunits. ^g ? means uncertain, not measured or not determined. ^h *E. coli*: *Escherichia coli*; *B. subtilis*: *Bacillus subtilis*; *S. cerevisiae*: *Saccharomyces cerevisiae*; *U. maydis*: *Ustilago maydis*; *E. gracilis*: *Euglena gracilis*; *P. polycephalum*: *Physarum polycephalum*; *T. pyriformis*: *Tetrahymena pyriformis*; *D. discoideum*: *Dictyococcum discoideum*; *D. melanogaster*: *Drosophila melanogaster*; *X. laevis*: *Xenopus laevis*.

DNA transactions with a single polypeptide chain (109,000 daltons in the case of DNA polymerase I)^{1,138,161}. The enzyme has different binding sites for DNA, for nucleoside monophosphates, and for nucleoside triphosphates. Furthermore, some of these enzymes combine various enzymatic functions in one polypeptide, namely polymerization in the 5'-3' direction^{45,46}, exonucleolytic activity in the 3'-5' direction (proofreading¹⁴²) exonucleolytic activity in the 5'-3' direction (excision, nick translation¹³⁹), pyrophosphorolysis¹⁴³, and pyrophosphate exchange¹⁴³. Some of these associated activities appear to contribute important functions in replication and repair¹⁶¹. Various associated activities were found in many bacteriophage and bacterial DNA polymerases (table 1 and references therein). There exists convincing evidence today that DNA polymerases from lower eukaryotes^{67-69,145,146} and even from higher eukaryotes such as mammals^{44,91,97,153,154} may possess at least one additional enzymatic activity beside the polymerization capacity (table 1).

Exonuclease working in the opposite direction of polymerization (so-called proofreading activity) have been reported in the lower eukaryotes *Ustilago maydis*¹⁴⁶, *Saccharomyces cerevisiae*^{67,68} and *Euglena gracilis*⁶⁹ as well as in higher eukaryotic mammalian cells^{91,101}. In the last case this enzyme is called DNA polymerase δ ⁴⁴. This activity might either be a distinct enzyme species or a special form (precursor?) of DNA polymerase α . DNA polymerase α , the putative vertebrate replicase (see next section), is reported to carry out pyrophosphate exchange and pyrophosphorolysis¹⁵³ and to possess exonucleolytic activity in the 5'-3' direction⁹¹, but one should bear in mind that this enzyme, even when purified to apparent homogeneity, has always 2 or more bands under the denaturing conditions of SDS-polyacrylamide gel electrophoresis. It appears that in higher eukaryotes the associated nuclease activities are in distinct polypeptides. Indeed, a variety of enzymes exonucleolytically active in the 5'-3'^{212,213} or 3'-5'²¹⁴⁻²¹⁶ direction have been isolated and characterized.

d) *Escherichia coli* polymerase compared with vertebrate DNA polymerase

Simple, single cell prokaryotes and mammalian cells alike possess at least 3 DNA polymerases. It is therefore tempting to speculate about analogous functions. From all the comparisons in table 2 a certain number of similarities between DNA polymerase III of *E. coli* and DNA polymerase α is evident. Inhibitor and functional studies further substantiate this (see next section). Properties such as molecular weight, K_m for deoxynucleoside triphosphates, pH optimum, inhibition by salts and sulfhydryl (-SH) blocking agents, template utilisation, biological functions, occurrence

of a holoenzyme form and stimulation by homologous singlestranded DNA binding proteins are similar. One should bear in mind that many of these results were obtained in a variety of laboratories under different assay conditions.

Mitochondria have similarities to prokaryotic organisms²¹⁷. These organelles harbor a small circular DNA^{218,219} and their own DNA polymerase γ ^{100,160,260}. A comparison of the γ polymerase with either DNA polymerase I or II suggests many similarities. Whether this coincidence is fortuitous or reflects true similarity cannot be decided on the basis of such a simple comparison.

2. Discovery of biological functions

DNA polymerases play a central role in 3 important biological events, namely DNA replication, DNA repair and DNA recombination. With the discovery of several distinct DNA polymerizing enzymes in a single cell authors began to ascribe one or the other of these events to certain DNA polymerases. In this section I try to compare the progress in this field in *Escherichia coli* and higher eukaryotes, mainly mammals.

a) Genetic approaches mainly in prokaryotes

After the discovery of the *Escherichia coli* DNA polymerase (later called DNA polymerase I) it was assumed that this enzyme was the replicase and would also function in processes like repair and recombination². However with the discovery of a viable, temperature sensitive mutant able to grow at low but not at higher temperatures, and which was severely deficient in this DNA polymerase²²⁰ this view changed completely. Despite extremely low levels of DNA polymerase, this mutant (*polA*) synthesized its DNA irrespective of temperature at the same rate as the wild type. Owing to improvements in assaying crude extracts other DNA polymerases were soon discovered in *polA* mutants. They were isolated and called in order of their discovery DNA polymerase II³⁵⁻³⁷ and DNA polymerase III^{38,39}.

After screening all the known DNA replication mutants for in vitro complementation of a receptor extract from a defective mutant with isolated wild-type DNA polymerases II and III it was found that *dnaE* (*polC*) was the genetic locus for DNA polymerase III²²¹. Thus it was established that DNA polymerase III was one of the essential enzymes in bacterial DNA replication. What is the function of the other 2 enzymes?

DNA polymerase I was shown to be involved in a variety of biological functions (see Kornberg¹ for details). 1. Involvement in chromosomal DNA replication^{161,222-224}: DNA polymerase I removes the RNA primer from the nascent DNA fragments (Okazaki

fragments²²⁵) with its 5'-3' exonuclease function and then fills the gaps by polymerization; the pieces are finally joined together by an enzyme called DNA ligase²²⁶. In addition it has been discovered that DNA polymerase I or a DNA polymerase I-like enzyme can abolish the temperature-sensitivity of a DNA polymerase III (*polC*) mutant^{232,233} suggesting a surrogate function of DNA polymerase I in replication.

2. Involvement in extrachromosomal DNA replication²³⁴: DNA polymerase is the replicase of certain plasmid DNAs (e.g. Col EI). 3. Involvement in excision repair of damaged bases²²⁷⁻²³⁰: mutants in DNA polymerase I are more sensitive to UV and X-ray irradiation²²⁷⁻²²⁹ and to treatment with the base altering agent methylmethane sulfonate²³⁰ suggesting an important role in restoring functionally damaged DNA. 4. Involvement in repair of misincorporated bases²³¹: it was found that DNA polymerases I and III are both necessary for this process and 5. involvement in recombination³¹⁵: DNA polymerase I has been found to be necessary for transposition.

The function of DNA polymerase II is obscure. *PolB* mutants defective in this enzyme were obtained by heavily mutagenising *polA1* cells²³⁵. These mutagenized cells grew normally at 25 and 42 °C and supported the growth of all known phages. Reports concerning DNA polymerase II mutants in DNA repair and recombination are controversial²³⁵⁻²³⁷. The single-stranded DNA-binding protein (SSB) of *E. coli*, which is essential for chromosomal replication²³⁸⁻²⁴⁰ stimulates DNA polymerase II¹⁸⁰ giving circumstantial evidence of a role in DNA replication.

DNA polymerase III is essential for chromosomal²²¹ and bacteriophage²⁴¹ replication. Furthermore, participation in excision^{3,236,242,243} and postreplication repair²³¹ mechanisms have been reported.

In summary, *Escherichia coli* polymerases seem to have more than one in vivo function. DNA polymerase III is the major replicase but also assists in DNA repair. DNA polymerase I is the major repair enzyme but also functions during replication as a gap-filling enzyme; apart from that it fulfills other essential replication tasks. Much more work is necessary to establish the role(s) of DNA polymerase II.

b) Genetic 'surrogate' systems mainly in eukaryotes

The rapid rise of interest in the DNA polymerases of lower and higher eukaryotes has led to a good knowledge of the physical, chemical and catalytic properties of the least 3 DNA polymerases, named α , β and γ ^{5-16,163}. In contrast, identification of their respective functions has lagged behind. This was mainly due to the lack of eukaryotic mutants defective in DNA replication. Indirect evidence is available which suggests the involvement of one or the other enzyme in a particular DNA transaction. A few years ago, a

replication mutant in the smut fungus *Ustilago maydis* was found to have a defective DNA polymerase¹⁷; this was followed recently by reports of DNA polymerase mutants in higher eukaryotes such as the fly¹⁸ and possibly in mammalian cells²⁴⁴. Each of the 3 DNA polymerases α , β and γ and their putative involvement in different DNA events will now be discussed in more detail.

DNA polymerase α is believed to play a major role in DNA replication. For a long time its localization provoked a lot of controversy, but by using refined extraction methods several authors showed the nuclear localization of this putative replicase²⁴⁵⁻²⁴⁸. Recent immunocytochemical methods, however, seem to indicate that the α polymerase might be localized in the perinuclear region of the cytoplasm²⁴⁹. First evidence for an involvement of this enzyme in DNA replication came from studies with cells that were stimulated to divide. Examples include liver regeneration after partial hepatectomy and stimulation of lymphocytes with phytohemagglutinin. Such cells had an increase of DNA polymerase α which paralleled the rate of DNA synthesis^{131,133,250,251}. These studies were followed by using synchronized cells where the α -polymerase was enhanced in the late G1 and early S phases of the cell cycle^{126,127}. DNA polymerase levels in physiologically differentiating and growing cells, such as neurons, spleen and muscle cells, showed an excellent correlation of stopping and onset of DNA replication with the decrease and increase of DNA polymerase α ^{128,129,252,253}. Isolation of replicating DNA further illuminated the function of α -polymerase. Replicating Simian virus 40 DNA^{118,254-256} (the small genome of Simian virus is replicated in nuclei of mammalian cells and, with the exception of the virus coded T antigen²⁵⁷, relies entirely on the cellular DNA replication machinery), replicating adenovirus DNA²⁵⁸⁻²⁶² (adenovirus DNA replication is dependent on the cellular DNA polymerases, but the viral DNA codes for several proteins that are involved in its own replication²⁶³), and isolated replicating chromatin from mammalian cells²⁶⁴ all have an associated DNA polymerase α . In vitro replication of parvovirus DNA is also dependent on the α -enzyme^{265,266}, and gene amplification during *Xenopus laevis* development appears to rely on DNA polymerase α ²⁶⁷.

DNA polymerases are unable to initiate de novo DNA synthesis in the absence of a 3'OH primer terminus. Short fragments of RNA serve as primers for DNA synthesis in vivo²⁶⁸. They are generated by RNA polymerases or by special primases which thus fulfil this function in a putative replication complex¹. DNA polymerase α is the only mammalian enzyme that is able to mimic in vivo DNA replication by elongating such RNA primers¹⁸⁶. DNA polymerase α is furthermore able, probably as a DNA polymerase

holoenzyme, to elongate in vitro long single-stranded primed DNAs^{25, 269-272}. Stimulation of DNA polymerase α by single-stranded DNA-binding proteins, and other factors including the interaction between structural proteins and the replicase at the replication fork will be discussed later. Adenovirus replication is the best characterized eukaryotic DNA replication system functioning in vitro; with this system, adenovirus DNA is fully replicated with partially purified proteins, and among them is DNA polymerase α ²⁷³ or a specially modified DNA polymerase α form³⁷⁸. Finally, there are a few reports claiming that the α -polymerase acts not only in replication but in addition has functional responsibilities in excision repair mechanisms^{274, 275}.

DNA polymerase δ displays similarity to DNA polymerase α ^{101, 154}. The novel and exciting finding is that this enzyme has a putative associated 3'-5' exonuclease proofreading activity. Whether this enzyme is a precursor form of DNA polymerase α or whether it is a distinct enzyme which serves a special function cannot be decided yet.

The function of DNA polymerase β was obscure for a long time. It was considered to be a repair enzyme (for a review of DNA repair and enzymes involved see Hanawalt et al.²⁰), more because of a need for a repair DNA polymerase, in analogy to bacteria, than on a basis of fact. No fluctuations can be measured during the cell cycle^{126, 127, 131, 133, 250, 251} or in physiological development^{128, 129, 252, 253}. In phytohemagglutinin-stimulated lymphocytes the major increase in DNA polymerase β was found to coincide with the peak of DNA repair activity and minimal DNA replication¹³⁵. Later it became evident that the β -polymerase can participate in excision repair of UV-damaged DNA of non-dividing neurons^{158, 207} that do not contain DNA polymerase α ¹²⁸. These results were confirmed in a variety of other systems^{199, 276-279}. Finally, with an elegant in vitro system where UV-treated DNA which had been incised with *Micrococcus luteus* correndonuclease²⁰⁰ was used, evidence was provided that DNA polymerase β is indeed able to use such an incision as a target to fill gaps and to repair the DNA in the 'patch and cut mechanism'¹⁹⁹. DNA polymerase α , on the contrary, is unable to do this. All these facts, together with many catalytic properties of DNA polymerase β ^{184, 185, 197, 198}, indicate that DNA polymerase β is involved in DNA repair.

On the other hand, it seems that at least in vitro DNA polymerase β is able to participate in DNA elongation. Elongation of DNA primed Φ X174 DNA^{270, 271} and in vitro replication of adenovirus DNA with isolated proteins need, in addition to DNA polymerase α , the β -enzyme as well²⁷³. These properties are reminiscent of DNA polymerase I, which is also a multifunctional enzyme in DNA synthesis¹⁶¹.

Following the discovery of DNA polymerase γ ⁴³ it took more than 5 years to realize that the mitochondrial DNA polymerase is a γ -type enzyme^{100, 160, 206}. An in vitro DNA replication system was developed using synaptic end knobs from neurons²⁸⁰. They contain mitochondria completely unassociated with nuclei, and these particles (called synaptosomes) can be isolated surrounded by a sealed membrane. Thus mitochondria of high integrity and purity could be obtained. These mitochondria carried out replicative DNA synthesis in vitro^{207, 253}, probably catalyzed by γ -polymerase which was the only DNA polymerizing enzyme present. The claim that aphidicolin, a potent inhibitor of DNA polymerase α (see later), has an effect on mitochondrial DNA replication²⁸¹ has recently been ruled out²⁸², which suggests that γ -polymerase is the only replicase in mitochondrial DNA.

DNA polymerase γ does not only occur in mitochondria, it is also present in the nucleus. The nuclear γ -polymerase is involved in adenovirus²⁹³ and parvovirus single-strand to double-strand replication²⁶. In both systems it seems, however, that DNA polymerase α ²⁵⁸⁻²⁶², and in adeno-replication even DNA polymerase- β ²⁷³, have certain roles in viral replication. Both mitochondrial²⁸³ and adenovirus²⁶³ DNA replication proceed in a continuous fashion, called strand displacement synthesis. The participation of γ -polymerase in strand displacement synthesis has been proposed¹⁴. Strand displacement synthesis needs a DNA polymerase that translocated in a non-distributive (processive) manner along the mother template. This is indeed the case for γ -polymerase⁸⁷.

What is the function of nuclear γ -polymerase besides replication of 'parasites'? Speculations could be a) a so far unknown function in nuclear DNA replication, b) involvement in amplification of genes or c) participation in recombinational events. In synchronized HeLa cells DNA polymerase γ in nuclei and cytoplasm increases more than twofold in early S-phase¹²⁶.

Summarizing all the evidence available for the functions of these 3 polymerases, I conclude that DNA polymerase α is the replicase for the chromosomal DNA; DNA polymerase δ , if it is indeed a distinct species, may help the α -enzyme by proofreading during replication. DNA polymerase γ is responsible for strand displacement synthesis in mitochondria and in certain viruses (e.g. adenovirus and parvovirus) and may have some specialized functions in chromosomal DNA synthesis like gene amplification or recombination. Finally, strong evidence is available that DNA polymerase β appears to be the excision-type repair enzyme.

c) Inhibitors and effectors

Inhibitors of DNA polymerases will be discussed exclusively in connection with selective inhibition of different enzymes and not with respect to their

Table 2. Comparison between *Escherichia coli* and vertebrate DNA polymerases^a

Property	<i>Escherichia coli</i> DNA polymerase			Vertebrate DNA polymerase		
	I	II	III ^b	α^c	β	γ
Physico-chemical						
Molecular weight, native	109,000	120,000	185,000;500,000	190,000-450,000	40,000	180,000-330,000
Molecular weight, denatured ^d (dl)	109,000	120,000	140,000	120,000-180,000	40,000	47,000
Catalytic subunit (d2)	109,000	120,000	140,000	120,000-180,000	40,000	47,000;160,000
Homogeneity	+	+	+	+	+	+
Isoelectric point	Acidic	Acidic	Acidic	Acidic	Acidic	Acidic
Order of elution from phospho-cellulose	2	3	1	2	1	3
Catalytic						
Specific activity of the purest preparations ^e	360,000	5,300	192,000	800,000	200,000	600,000
K _m for deoxynucleosidetriphosphates	Low	Low	High	High	High	Low
pH optimum	7.0	7.5	7.2	7.2	8-9	8-9
Effect of ionic strength (100 mM KCl) ^f	^f Stimulation	Slight inhibition	Strong inhibition	Strong inhibition	Stimulation	Slight stimulation
Effect of sulphhydryl (-SH) blocking agents	No effect	Inhibition	Inhibition	Inhibition	No effect	Inhibition
Divalent kation optimum (mM)						
Mg Cl ₂	2	10	6-15	2-7	5-25	5-12
Mn Cl ₂	0.1	0.5	1	0.5	0.3-0.8	0.1-0.6
Functional and biological ^g						
Template utilization						
ss DNA	-	-	-	-;(+)	-	-
ds intact DNA	-	-	-	-	-	-
ds extensively nicked ^h DNA	+	+	+	+	+	+
ds gapped DNA:						
gaps of < 10	+	-	-;-	-	+	?
gaps of 10-100	+	(+)	+;(+)	+	(+)	+
gaps of > 100	+	-	-;+	-;+	-	+
DNA primed ssDNA ⁱ	+	(+)	-;+	-;+	(+)	+
RNA primed ssDNA ⁱ	+	(+)	-;+	-;+	-	-
Synthetic homopolymer DNA ^j :						
d template · d primer	+	+	+	+	+	+
d template · r primer	(+)	?	+;+	+	-	(+)
r template · d primer	+	?	-;-	-	(+)	+
Main biological function:						
DNA replication	(+)	?	+	+	?	+
DNA repair	+	(+)	(+)	?	+	?
Polymerization: 5' → 3'	+	+	+	+	+	+
Exonuclease : 5' → 3'	+	-	+	-;(+) ^k	-	?
Exonuclease : 3' → 5'	+	+	+	-;(+)	-	?
Pyrophosphorolysis	+	+	+	+	-	?
Pyrophosphatexchange	+	+	+	+	-	?
Relative amount in growing cell %	> 90	4-5	2-3	> 80	10-15	2-5
Number of molecules per cell	400	17	10	6 × 10 ⁴	2.5 × 10 ⁴	4 × 10 ⁴
Number of molecules per <i>Escherichia coli</i> genome size ^l	400	17	10	600	250	400
Polymerization rate (nucleotides × sec ⁻¹ × enzyme ⁻¹)	10-20	10-15	100-150; > 300	30	2.5	32
Processivity (nucleotides × enzyme ⁻¹)	188	?	10-15; > 5000	9-17	1	4000
Accuracy	1.6 × 10 ⁻⁶	?	5 × 10 ⁻⁷	8 × 10 ⁻⁵	3 × 10 ⁻⁵	> 10 ⁻⁴
Strand displacement synthesis	+	-	-	-	+	+
Occurence of holoenzyme form	-	?	+	+	-	-
Effect of homologous single-stranded DNA binding protein	No effect	Stimulation	Inhibition; Stimulation	Stimulation	Stimulation	?
Effect of histones:						
core histones	Inhibition ^m	?	Inhibition ^m	Inhibition	Inhibition	Inhibition
H1 histones	Inhibition ^m	?	Inhibition ^m	Inhibition	Inhibition	Stimulation

^a Data were collected from literature references 1, 45, 46, 142, 143, 164-174 for DNA pol I; 1, 35, 38, 47-49, 175-180 for DNA pol II; 1, 22, 50-54, 102, 104, 122, 125, 144, 176, 181-183 for DNA pol III; 11, 89, 91, 93-95, 125, 184-196, 210, 211 for DNA pol α ; 11, 86, 96, 97, 155-158, 167, 184, 195-202 for DNA pol β ; and 10, 11, 87, 88, 98-100, 160, 203-207 for DNA pol γ . ^b If there are 2 numbers or statements, the first stands for the DNA polymerase III core enzyme and the second for the DNA polymerase III holoenzyme. The parameters were determined with specific assays. ^c If there are 2 numbers or statements, the first stands for DNA polymerase α and the second for a DNA polymerase α multipolypeptide complex ('holoenzyme or holoenzyme fragment'). ^d Determined after SDS-polyacrylamide gel electrophoresis either by a conventional staining method (dl) or by activity measurements after renaturation (d2) of the enzyme in the gel (see ref. 124 for explanation). ^e Specific activity is expressed as units × mg⁻¹. One unit is defined as the incorporation of 1 nmol total deoxynucleoside triphosphate into acid insoluble material in 60 min at 37 °C. ^f Compared to an assay in the absence of any salt. ^g The minus sign means absolutely negative; the plus sign positive and the plus sign in parentheses either poorly positive or moderate evidence. A question mark

Table 3. Inhibitors of DNA polymerases

	DNA polymerase Prokaryote			Eukaryote		
	I	II	III	<i>a</i>	<i>β</i>	<i>γ</i>
Arabinosyl NTP's	− (117) ^a	++ (117)	+ (177)	++ (288)	− (289)	− (288)
Dideoxy NTP's	− (1)	++ (1)	++ (1)	− (118)	++ (118)	++ (118)
N'ethylmaleimide	− (1)	++ (1)	++ (1)	++ (7)	− (7)	++ (7)
Aphidicolin	− (286)	− (286)	− (286)	++ (119)	− (119)	− (119)
Phosphonoacetate	− (287) ^b			++ (120)	− (120)	− (120)

++ , Strong inhibition; + , slight inhibition; − , no inhibition. ^aReferences numbers in brackets. ^b*M. luteus* DNA polymerase I.

mechanism of action on the enzyme itself (for detailed mechanisms for inhibition of DNA polymerases see Brown and Wright²⁸⁴ and Cozzarelli²⁸⁵. Inhibitors have been extremely useful in elucidating DNA polymerase functions, especially in vertebrates and mammalian cells.

Table 3 outlines the effects of known DNA polymerase inhibitors of the following 5 types: arabinofuranosyl-nucleoside triphosphates, 2',3'-dideoxynucleoside triphosphates, the sulfhydryl blocking reagent N-ethylmaleimide, the tetracyclic diterpenoid aphidicolin²⁹⁰⁻²⁹² and phosphonoacetic acid. DNA polymerase *a* is inactivated by all inhibitors except 2',3'-dideoxynucleoside triphosphates. In contrast, 2',3'-dideoxynucleoside triphosphates inhibit DNA polymerase *β* and *γ*. These two enzymes can then be distinguished by the resistance of the *β*-polymerase to high concentrations (10 mM) of N-ethylmaleimide. By working with these substances it has been demonstrated that the *a*-polymerase functions in chromosomal^{119,294-305} and viral (papova^{118,256,298} or adenovirus^{262,306-308}) DNA replication. It has been similarly shown that the *γ*-enzyme is solely responsible for mitochondrial DNA replication^{207,282} and, together with *a*-polymerase, is involved in adenovirus^{261,293,301,307} but not in papovavirus replication²⁹⁸. The data obtained with these inhibitors were all in agreement with the results of the approaches mentioned in the previous section.

The uncommon purine nucleotide diadenosine 5',5'''-p¹,p⁴-tetrphosphate (Ap₄A) has been found to be a trigger of DNA replication in mammalian cells^{309,310}. The target of this molecule seems to be the DNA polymerase *a* holoenzyme^{311,312}. This target is lost in postmitotic cells again suggesting a role for DNA polymerase *a* as the replicase⁹². The effect of Ap₄A seems to be ubiquitous since it also binds to *Escherichia coli* DNA polymerase III holoenzyme³¹³ and to *Physarum polycephalum* DNA polymerase³¹⁴.

d) Comparative summary

Comparison of prokaryotic and eukaryotic DNA polymerase functions manifests that each enzyme seems to have more than one particular role in DNA transactions, probably in collaboration with subunits or auxiliary proteins. Taking into account all the physico-chemical, biochemical and biological comparisons the following statements are permissible (see tables 1-3): DNA polymerase *a* is analogous to DNA polymerase III, DNA polymerase *β* can be likened to DNA polymerase I, and DNA polymerase *γ* resembles to some extent DNA polymerase II.

3. Proteins interacting with DNA polymerases

With the discovery and description of multienzyme systems involved in *Escherichia coli* replication, using the small bacteriophages Φ X174, G4 and M13^{316,317} as model replicons it became evident that the in vitro DNA elongation step needs DNA polymerase III too. Soon it was realized that it must be a complex form of this polymerase. Such a multipolypeptide aggregate was isolated and called DNA polymerase III holoenzyme²⁴¹. This DNA elongation complex is required for elongation of primed single-stranded DNA³¹⁶ and for specifically nicked double-stranded Φ X174 DNA³¹⁸, while on nicked or gapped DNA's the DNA polymerase core alone is active⁵¹⁻⁵⁴. Such artificial templates are DNAs of any source that have been partially digested with a deoxyribonuclease in order to create gaps containing a free 3'OH group as primer for the enzyme.

What functions do these additional factors, called holoenzyme subunits or DNA polymerase accessory proteins, provide during the elongation step? It is essential for the DNA elongation machinery to synthesize the daughter strands efficiently and accurately. Interaction at the primer terminus with the primosome¹⁰⁶ or recognition of the termination site(s) is

indicates that a property is not known or was not measured with homogeneous enzymes. ^h Optimal template activity is reached after extensive treatment with DNase I, when 5-20% of the DNA was rendered acid soluble. Such digested DNA contains structures that are similar to gapped DNA with random gap sizes. ⁱ A primed single-stranded DNA is a long single strand that contains a short complementary nucleic acid strand annealed to it. Examples are: Multi- or singly-primed ss bacteriophage DNA's such as ϕ X174, G4 or M13 or, as an in vivo example, parvovirus DNA. ^j d: deoxy; r: ribo ^k See Chen et al.⁹¹. ^l The vertebrate genome is for this purpose assumed to be 1000 times larger than that of *Escherichia coli*. ^m Tested either with the eukaryotic histones or with a prokaryotic histone-like protein^{208,209}.

possible. In this chapter proteins interacting with DNA polymerases are introduced and compared. Table 4 gives an overview of the structure and function of replicative DNA polymerases from *Escherichia coli*, *Drosophila melanogaster* and calf thymus. It is evident that much more functional information is available for the prokaryotic than for the eukaryotic systems, because of the advantages mentioned previously. In what follows I would like to present four replicases and their subunits in more detail.

These are: T4 DNA polymerase, *Escherichia coli* DNA polymerase III, *Drosophila melanogaster* DNA polymerase α and mammalian DNA polymerases α . Bacteriophage T4 DNA polymerase (gene 43 protein)³²⁶ needs 4 additional proteins in order to elongate a primed T4 DNA²⁴. One is the single-stranded DNA binding protein encoded by gene 32³²⁷ and the others are the three accessory proteins specified by genes 45, 44 and 62²⁴. This elongation machinery proceeds at an extremely fast rate³²⁸ and is highly accurate³²⁹. The gene 44/62 complex possesses a DNA-dependent ATPase (or dATPase) activity³³⁰ and binds directly to DNA³³¹, while 45 protein serves as a linker between the polymerase and the 44/62 replication complex³³¹. These proteins help the DNA polymerase to pass hairpin loops efficiently and also act as a 'sliding clamp' to enhance the binding of the polymerase to the 3'OH primer terminus, thus making the polymerase more processive and accurate³³¹ (see also next section).

DNA polymerase III holoenzyme of *E. coli* has an even more complex structure (table 4) than the T4 elongation apparatus. While the latter cannot be isolated as a entity from bacteriophage T4 infected cells³³², it is possible to purify a DNA polymerase III holoenzyme several thousand-fold as a stable multi-polypeptide complex²². The DNA polymerase III core

enzyme contains three polypeptides called α , ϵ and θ of 140,000, 25,000 and 10,000 daltons, respectively⁵⁴. In addition there are at least 5 polypeptides associated with highly purified enzyme preparations. These are: the β -subunit of 37,000 daltons¹⁰³, the γ -subunit of 52,000 daltons¹⁰⁴, the δ -subunit of 32,000 daltons¹⁰², the ζ -subunit of 48,000 daltons³²⁴ and finally the τ -polypeptide, a 83,000 daltons DNA-dependent ATPase³²⁵. As far as the genetic loci are concerned, the *dnaE* gene codes for the α -subunit³²², the *dnaN* for the β -subunit¹⁰⁵, the *dnaZ* for the γ -subunit¹⁰⁴, and *dnaX* for the δ -subunit¹⁰². The *mutD* gene might specify one of the small core subunits ϵ or θ ³²³. The α -subunit is the catalytic DNA polymerizing activity^{122,125}, the β -subunit transfers the holoenzyme to the primers or to the primosome^{102,105,333}, the γ - and δ -subunits might be involved in processivity and fidelity events^{182,183} as well as in the interaction with the 3'OH primer³³³. The DNA-dependent ATPase activity of the τ polypeptide is not required for in vitro replication of small bacteriophages but might have analogous function to the 44/62 proteins in T4 replication³³¹, namely to make the DNA polymerase more processive³³⁴ (see also next section). The energy derived from ATP hydrolysis by the τ protein may be used to activate the holoenzyme complex³³⁵. The *E. coli* DNA elongation factors I, II and III^{23,336} could have the following relationship to the holoenzyme subunits: factor I might be identical to the β -subunit, factor II to a mixture of γ - and δ -subunits and factor III to the δ -subunit alone^{102,104}. Finally it should be mentioned that the single-stranded DNA binding protein is required for in vitro³³⁷ and in vivo²³⁸ elongation of *E. coli* DNA.

The best-characterized eukaryotic replicative DNA polymerase holoenzyme is the α polymerase from *D. melanogaster* (table 4). It has been purified to homogeneity⁷⁵ and it consists of at least 4 subunits

Table 4. Functional roles of prokaryotic and eukaryotic DNA polymerase subunits

<i>E. coli</i> DNA polymerase III holoenzyme (144, 319)				<i>Drosophila melanogaster</i> DNA polymerase α (75, 123)			Calf thymus DNA polymerase α (92, 94, 95, 124, 125, 320)	
Subunit	kdaltons	Genetic locus	Function	Subunit	kdaltons	Function	kdaltons	Function
α	140	<i>dna E</i> (122)	Catalytic subunit (125, 322)	α	148	Catalytic subunit (123, 125)	210-230 158, 155, 148	} Precursor? (125)
β	37	<i>dna N</i> (105)	Transfer of holo-enzyme to primo-some (105, 322)	β	58	?	134, 125, 110 105, 100 90, 74	Catalytic subunit ? ?
γ	52	<i>dna Z</i> (104)	Interaction with primosome, processivity, fidelity (104, 182, 183)	γ	46	?	70-50 ^a 57	? A _{p4} A binding protein (92, 321)
δ	32	<i>dna X</i> (102)		σ	43	?		
ϵ	25	one <i>mut D</i> ? (323)	Part of DNA polymerase III core (54)				50-40 ^a 25 ^b	? ?
θ	10							
ζ	48	?	? (324)					
τ	83	?	DNA dependent ATPase (325)					

^aSeveral bands each; ^bfrom HeLa cells 272. References numbres in brackets.

called α , β , γ and δ of 148,000, 58,000, 46,000 and 43,000 daltons, respectively^{76,123}. The term holoenzyme should be taken with some caution, since the enzyme has not been isolated with an elongation assay similar to *E. coli* and it might therefore be a fragmentary complex. The same statement is also valid for mammalian DNA polymerases α (discussed below). Indeed, if a single RNA primed single-stranded M13 template is elongated with the *Drosophila melanogaster* α -enzyme the polymerisation rate is an order of magnitude lower than that of *Escherichia coli* DNA polymerase III holoenzyme²⁵. The α subunit is the catalytic polypeptide^{25,125}, while the functions of the other 3 proteins are unknown. It remains to be seen whether the *Drosophila* replicase is more complex when it is isolated with an elongation assay. The mammalian replicase, DNA polymerase α , seems to be an extremely complex entity. It is impossible to give a conclusive picture about the structure of this enzyme (complex) at this time. In table 5 some of the purest mammalian DNA polymerases α are compared to homogeneous replicases from other organisms. All these complexes are pure in the sense that one stained band on a native polyacrylamide gel coincides with the polymerase activity. However, after denaturation and SDS-polyacrylamide gel electrophoresis, they show 2 or more polypeptides. What does this imply? Isolation of holoenzyme forms or fragments might be a likely explanation. Depending on the tissues and isolation assay more or less complex structures might have been isolated. One has to perform the isolation with specific elongation assays in order to solve this question. Indeed, if a ss Φ X174 DNA is used as a template with a complementary restriction fragment as a primer and a partially purified DNA polymerase α as the enzyme, an α polymerase stimulatory protein can be isolated²⁷⁰. It is known from work with *Escherichia coli* DNA polymerase III holoenzyme that

multiple peaking on affinity or ion exchange columns are due to different forms or fragments of a holoenzyme, e.g. the presence or absence of an additional protein in the DNA polymerase complex^{319,322}. Different holoenzyme forms could be one of the reasons for different findings and for the postulation of different DNA polymerase α forms^{339,340}. Proteolysis and different physiological complexes are other possible explanations for conflicting reports (see e.g. Albert et al.³²⁰ and later). From table 5 it seems that high-molecular-weight (200,000 dalton) complexes of DNA polymerases α are found in all cases. On the other hand, on denaturing gels, a high-molecular-weight subunit ($\geq 125,000$ dalton) often appears when protease inhibitors are used during the isolation procedures. Applying a recently described technique to detect DNA polymerase activities after SDS gel electrophoresis¹²⁴ it could be demonstrated that a high-molecular-weight polypeptide ($\leq 125,000$ dalton) is responsible for chromosomal DNA replication both in lower and higher eukaryotes¹²⁵. Furthermore it is evident that intermediate (75,000–110,000 dalton) and low-molecular-weight (35,000–75,000) active fragments are detected if extraction and isolation are performed under conditions where proteolysis is allowed to occur¹²⁵. Comparing the *Escherichia coli* DNA polymerase III holoenzyme subunits with the different polypeptides reported to occur in calf thymus DNA polymerase α it is striking that almost all reported DNA polymerase III holoenzyme subunit molecular weights can be identified in DNA polymerase α preparations of different authors (tables 4 and 5). Another DNA polymerase α subunit which has been characterized is the 57,000 dalton protein, the binding target for the small-molecular-weight DNA replication trigger Ap₄A^{92,321}. Association of DNA polymerase α with tryptophanyl-tRNA synthetase³¹¹

Table 5. Subunit structure of apparently homogeneous replicases

Source	Molecular weight $\times 10^{-3}$			Denatured	Protease inhibitor	Reference
	Native Electrophoresis	Filtration	Sedimentation			
Human ^a	265–280	140	140	(150–170), 76, 66	–	89
Calf thymus ^a	410	400	250	230–210, 155, 140, 103, (70–50)	–	90, 339
				(125) ^b , 64, 63, 62, 60, 57, 55, 52	–	92
				125 ^b , 105, 100, 95, 64, 63, 62, 60, 57, 55, 52,	+	125
			250	(150), 134, 123, (70–40)	+	94
Rat ^a	1300			158, 148, 140, 134, 123, 110, 90, 74, 59, 55, 48, 30, 10	+	95, 272
Mouse ^a	450–525		190	156, 64, 61, 58, 54	+	93
<i>Drosophila mela-</i>				54, 47	–	91
<i>nogaster</i> ^a	550		280	148, 58, 46, 43	+	75
<i>Ustilago maydis</i>			180	55 ^b , 50	–	66
				200 ^b , 115, 68, 62	+	125
<i>Escherichia coli</i>	528–580 ^c		240–250 ^d	140, 83, 52, 48, 37, 32, 25, 10	–	22, 54, 324

^aDNA polymerase α .
^bUpper row stained bands only, lower row stained bands and activity bands, not seen upon staining, after renaturation (see ref. 124, 125 for explanation).
^cDNA polymerase III holoenzyme.
^dU. Hübscher, unpublished results.

might be of significance in this connection, since this enzyme, at least in vitro, is involved in the synthesis of Ap₄A in the backreaction of the amino-acid activation process³⁴¹.

A fair number of proteins that interact with DNA polymerase α have been described in recent years. Among these are homologous single-stranded DNA binding proteins^{269,342-349}, protein kinase³⁵⁰, DNA dependent ATPase³⁵¹, proteins that can convert the α -polymerase into another structural form³⁵², a basic non-enzymatic protein factor of 30,000 daltons³⁵³, and α -polymerase accessory proteins from HeLa²⁷² and rat giant trophoblast cells³⁵⁴. Similar proteins have been identified and isolated from *Tetrahymena pyriformis*³⁵⁵ and the sea urchin³⁵⁶. There exist also proteins which inhibit various DNA polymerase forms in different ways, e.g. ricin, hemin and non-histone chromosomal proteins³⁵⁷.

Finally it should be mentioned that stimulatory proteins have been described for DNA polymerase β , e.g. a heat stable single-stranded DNA binding protein³⁵⁸ or factor IV from hepatoma cells³⁵⁹, which is most probably a 5'-3' and 3'-5' exonuclease²¹⁵.

In summary, it may be stated that the proteins working with replicases during the elongation step are well characterized in prokaryotic systems, while in eukaryotes the knowledge is still fragmentary. It is therefore impossible to compare proteins with putatively similar roles. In conclusion, the DNA polymerase polypeptide alone is not able to elongate long stretches of DNA. Additional auxiliary subunits are needed to support the DNA polymerase in prokaryotes as well as in eukaryotes.

4. Action of replicative DNA polymerases and their associated proteins at the replication fork

In the previous section the DNA polymerase and its accessory subunits were discussed by themselves. It is, however, obvious that other proteins and cofactors have to be present at the replication fork in order to enable the replicase to perform its job.

DNA replication is controlled at the point(s) of initiation³⁶¹. After initiation events, e.g. recognition of specific DNA sequences by proteins such as *Escherichia coli* protein ν ³⁶² or gene A protein from Φ X174^{363,364}, formation of a prepriming complex³⁶⁵ and the synthesis of oligoribonucleotides by primases³⁶⁶, it is the task of the DNA elongation machinery to replicate the primed or specifically nicked DNA. Several events have to be considered: a) the unwinding of the double-stranded DNA in advance of replication, b) the continuous synthesis in the overall 5'-3' direction, c) the discontinuous synthesis in the 3'-5' direction, d) the translocation along the mother DNA and e) the degree of accuracy during polymerization.

It is beyond the purpose of this review to give detailed information on mechanisms of initiation. This has been reviewed³⁶¹ and detailed data on formation, movement and conservation of primosomes have been published^{19,106,107}. The functional interaction of the DNA elongation apparatus with primers and priming proteins during DNA synthesis will be described. That there are several possibilities for a replicase to start and proceed will come clear from the following discussion.

a) The unwinding reaction ahead of leading strand polymerization

Unwinding ('unzippering') of double-stranded DNA is necessary since most of the DNA polymerases are incapable of strand displacement. Exceptions are *Escherichia coli* DNA polymerase I¹, T5 DNA polymerase⁵⁸ and DNA polymerase γ ⁸⁷. The 2 prokaryotic model systems of bacteriophage T4 and bacteriophage Φ X174 again give us the best information in this field.

The T4 DNA polymerase gains limited displacement ability in the presence of the 'helix destabilizing protein' (gene 32 protein) even in the absence of ATP³⁶⁷. This capability is greatly enhanced by the three auxiliary proteins 45 and 44/62^{328,330,331,368}. It seems, but is not proven yet, that ATP is the energy source required for this process^{330,331,368}. The displacement mechanism seems to be coupled with DNA synthesis³⁶⁸. The same proteins seem to enhance the rate at which T4 DNA polymerase traverses helical regions in single-stranded templates³³¹. Whether T4 topoisomerase is involved in unwinding is not clear at this time³⁶⁹.

In vitro studies with *E. coli* DNA polymerase III holoenzyme are performed with circular viral DNA. Bacteriophage Φ X174 requires by far the largest number of proteins and is therefore considered to be the model system for *E. coli* chromosomal DNA replication^{121,317}. The conversion of single-stranded DNA to the double-stranded form (RF I) is an analogue for discontinuous replication on the lagging strand³⁷⁰, while synthesis of positive single-stranded viral DNA from RF I is the model for the continuous mode of replication on the leading strand³²⁵. The *rep* protein, a helicase, is necessary for the unwinding of this Φ X174 DNA³¹⁸ after a specific nick has been introduced by the Φ X174 encoded gene A protein³⁶³. This unwinding process can be uncoupled from replication^{371,372}. At the same time as unwinding takes place, the single-stranded regions have to be stabilized by the single-stranded DNA binding protein (SSB)³⁷². Finally the DNA polymerase III holoenzyme copies the genome concurrently with unwinding³⁷³. In addition, *E. coli* has several other helicases of which the in vivo functions are so far unknown³⁷⁴. Unwinding possibly

needs the participation of DNA topoisomerases of type II^{369,375}. One of these enzymes (*E. coli* DNA gyrase) can, among other reactions, introduce negative superhelical twists into closed circular DNA molecules with ATP as a cofactor³⁷⁵ and such turns may give the motive force for the unwinding of the double helix³⁷⁶. Nicking closing is another possible mechanism by which topoisomerases of type I can facilitate unwinding³⁷⁶.

Work in eukaryotes using model replication forks includes the previously mentioned papova-, parvo- and adenovirus systems. The former two have not been sufficiently fractionated and characterized to allow the attribution of functional roles to proteins other than the DNA polymerase core enzyme itself¹⁵. The adenovirus in vitro system, however, although it relies on a few adenovirus-encoded replication proteins³⁰ is being worked out in some detail. A protein fraction from adeno-infected HeLa cells is able to replicate in vitro adenovirus DNA in the presence of an adenovirus DNA binding protein²⁷³ and the 55,000 dalton protein which is covalently linked to the 5'-prime end of the DNA^{273,377}. This protein fraction was been purified, and found to contains DNA polymerase α , a DNA dependent and an independent ATPase activity²⁷³ and a functional precursor 80,000 dalton form of the covalently bound protein³⁷⁸. No nicking closing activity, DNA ligase, exonuclease, DNA gyrase or single-stranded DNA binding protein could be detected in these preparations. These viral systems are promising tools to elucidate DNA elongation mechanisms, because they rely completely on the host's enzyme machinery.

Apart from these viral studies, enzymes and proteins which probably help the replicase to function at the fork have been isolated and characterized. Among those are single-stranded DNA binding proteins³⁷⁹⁻³⁸² single-stranded DNA dependent ATPase^{382,383}- and topoisomerases of type I (nicking closing enzyme) or type II³⁸⁴.

b) Interaction with multiple primers in advance of and during lagging strand polymerization

Because DNA polymerases synthesize exclusively in the 5'-3' direction, one strand, the lagging strand, has to be synthesized discontinuously in small pieces, called Okazaki fragments²²⁵. Each piece has to be initiated individually, so that the replicase can elongate. The DNA elongation machinery has to interact frequently with the primers or the primosome¹⁰⁶. Again I would like to discuss, first, the more highly elaborated T4 and the *Escherichia coli* bacteriophage systems, and then at the end, to give the fragmentary data available for eukaryotes.

In T4 the seven proteins 32, 41, 44, 45, 61, 62 and 43 (DNA polymerase) can synthesize DNA in vitro dis-

continuously on the lagging side giving rise to Okazaki fragments of 10,000 nucleotides in length³²⁸ (see Liu et al.²⁴ for more details). This system, however, is incapable of removing the RNA primers, of sealing the Okazaki pieces together and of reinitiating replication forks at defined origins³²⁸.

The most elegant system by far for lagging strand synthesis is the conversion of single-stranded Φ X174 DNA to its double-stranded form³²⁵. The primosome¹⁰⁶, containing all proteins needed to form a primer at a defined place in the Φ X174 chromosome, moves after complex formation at the replication origin in the antielongation direction¹⁰⁷. This processive movement with the advance of the replication fork is an attractive proposal for repeated initiation of nascent (Okazaki) fragments¹⁰⁷. Thus, the DNA polymerase III holoenzyme always finds primers 'backwards' and starts to elongate as soon as it reaches the previous synthesized RNA primer. The *dnaN* gene product¹⁰⁵, which is the β -subunit of the holoenzyme, is responsible for the interaction with the primer synthesized by the *dnaG* primase³²² and probably also for the interaction with the primosome³²⁴. Thus it appears that this protein enables the replicase to find the primosome efficiently in order to carry out the concerted actions of frequent priming and elongation. In contrast to T4, in this Φ X174 lagging strand model small gaps can be filled in vitro, the primers removed by DNA polymerase I, and the remaining nick sealed by the action of DNA ligase³¹⁷.

In eukaryotes the systems are either not defined or not fractionated enough to permit comparisons. The DNA polymerase α of *Drosophila melanogaster* was tested on RNA primed M13 DNA²⁵. Using a multi-primed template the *Escherichia coli* single-stranded DNA binding protein enhanced the rate and extent of replication, while on singly-primed DNA, the same protein had no effect. The product size was 200-600 nucleotides in the absence and 2300-2400 in the presence of the binding protein. The catalytic α -subunit alone is unable to use such a template²⁵, which is very reminiscent of the DNA polymerase III core²². Thus it seems that the 3 additional DNA polymerase subunits may have functional responsibilities in the elongation process.

The primase described so far in mammalian cells³⁸⁵ was not pure enough for elongation studies. Artificially primed systems such as DNA or RNA primed single-stranded Φ X174 DNA^{270,271} give insight into some aspects of the elongation process. It seems, as in *E. coli*, where the concerted action of DNA polymerase III holoenzyme and DNA polymerase I is needed for a complete synthesis of an Okazaki fragment, that in eukaryotes as well 2 distinct DNA polymerases are required. RNA primers are elongated by DNA polymerase α to a certain extent^{186,270,271} and the β -enzyme is necessary for continuation^{270,271}. In

vitro elongation of initiator RNA in isolated nucleoprotein complexes from polyoma infected cells can be performed by DNA polymerase α and these in vitro synthesized Okazaki fragments can be joined by extracts of early *D. melanogaster* embryos³⁸⁸. All these results have to be interpreted with caution, since the DNA polymerases used were either not homogeneous or, most probably, were fragments of a holoenzyme. Even though it is speculative at this time, an attractive hypothesis is that 2 DNA polymerases share the work on the lagging strand: DNA polymerase III or γ does the major work of replication, while DNA polymerase I or δ , together with an associated or attached 5'-3' exonuclease²¹⁵, fills in the gaps and remove the primers.

c) Mode of translocation

Generally, a DNA polymerase can work in 2 different ways during elongation. In the 1st case the enzyme at the actual primer terminus binds a deoxyribonucleoside triphosphate, catalyses polymerization on a mother template and finally translocates to the next base without leaving the DNA. A 2nd, alternative mode is the release of the enzyme after each polymerization step. The former mechanism is called processive and the latter distributive. A 3rd mechanism has been proposed and called 'quasi' processive; it is somewhere between the two extremes¹⁹⁴. For reasons of economy, speed and fidelity (see below) one might postulate that a replicase should work in the processive mode of translocation.

Recently 2 methods to determine the degree of processivity have been worked out. The 1st¹⁶⁷ measures the rate of DNA synthesis in the presence of all four deoxyribonucleoside triphosphates versus the rate in the absence of one, two, or three of the four. If the reaction is carried out with excess template:primer, the number of nucleotides polymerized for each binding event involving the DNA polymerase and the template can be calculated. The 2nd method¹⁹⁴ uses the artificial template (dA)₃₀₀ · (dT)₁₀ as template/primer. The reaction is carried out under conditions, where most probably the enzyme uses the template/primer only once during a polymerization step. The product is then purified over an oligo(dT)-cellulose column, digested by micrococcal nuclease and spleen phosphodiesterase and the nucleosides and nucleotides separated. The degree of processivity can finally be calculated from the nucleoside/nucleoside × nucleotide ratio.

Before discussing the data it should be stated that reaction conditions such as DNA structure, ionic strength and temperature can affect processivity. In addition, as will become clear later, the source and particularly the purity of an enzyme can influence the results due either to 'intrinsic' nucleases or to factors and proteins that make the enzyme more processive.

The T4 DNA polymerase alone has a processivity of 3–12 nucleotides¹⁹⁴ and is alone only slightly or 'quasi' processive. If the accessory proteins 45 and 44/62 are added the DNA polymerase enhances its rate and becomes fully processive, that is several hundred nucleotides are added per binding event³⁸⁶. These proteins together with the DNA polymerase could build the DNA 'walking machine'³³¹. The ATPase of the 44/62 complex serves as the 'sliding clamp' and the 45 protein is essential for the formation and maintenance of this 'walking machine'³³¹.

In *Escherichia coli* most studies have been carried out with DNA polymerase I. Depending on the reaction conditions and on the assay the enzyme is highly processive (188 nucleotides at low salt and high temperature) or only 'quasi processive' (3.3 nucleotides at high salt and low temperature)¹⁶⁷. A 'quasi processive' value was obtained when an artificial template was used in the assay³⁸⁷. A mutant of DNA polymerase I (*pol* A5) has a 5-fold decreased processivity³⁸⁷. DNA polymerase III core enzyme has a processivity of 10–15 nucleotides¹⁸³. On the other hand, DNA polymerase III holoenzyme is extremely processive¹⁸³. On an artificial homopolymer or on a randomly primed template the processivity is more than 100 nucleotides. Using a single-stranded G4 DNA template that has been primed with the *dnaG* primase in the presence of SSB the holoenzyme can replicate the full genome with one binding event. Thus the processivity is greater than 5000 nucleotides. This experiment shows that in vivo-like replication systems are necessary to work out the true processive translocation. The DNA polymerase III holoenzyme subunits γ , δ and τ are the most likely candidates for functions analogous to those of the above-mentioned T4 proteins 45 and 44/62.

In lower eukaryotes, recent studies with the *Drosophila melanogaster* α -polymerase²⁵, using a multiprimed M13 DNA, demonstrate that the α -polymerase (α , β , γ and δ subunits) has a processivity of 14. The catalytic α -subunit alone, on the other hand, has one of only 5. Heterologous single-stranded binding proteins enhance the processivity more than 20 times to 285 for the 4 subunit α -polymerase, while the α -subunit alone in the presence of binding protein becomes fully distributive. It seems, as for T4 and *Escherichia coli*, that subunits other than the catalytic one serve as clamp holders for DNA polymerase at the replication fork.

In higher eukaryotes pure DNA polymerase α preparations are either distributive¹⁹³ or 'quasi' processive (11 nucleotides)^{188, 192–194}. Other forms of DNA polymerases α (more holoenzyme-like?) are more processive^{192, 406}. DNA polymerase β is found to be fully distributive^{167, 202} but a 'quasi' processiveness of 9 nucleotides has also been found¹⁹⁴. The γ -polymerase, as one expects for an enzyme that is capable

of strand displacement, is a highly processive enzyme⁸⁷. In conclusion, it seems likely that replicative DNA polymerases need additional proteins to become fully processive. This high processivity may be responsible for the fast rate at which replication proceeds in vivo.

d) Accuracy of polymerization

Accurate duplication of the genetic material in advance of cell division is a prerequisite for any living organism. DNA polymerases play a central role in the correct incorporation of a precursor nucleotide on a mother template. However, there are other factors, enzymes and structural proteins that influence the accuracy of replication. Several mechanisms support the high degree of fidelity which is estimated to be on the order of 10^8 – 10^{11} in vivo^{31,195,389}. These are: 1. the correct selection of the nucleotide by the enzyme at the 3'OH terminus of specific primers, 2. an editing mechanism to throw out incorrect bases as soon as they are incorporated (proofreading) and 3. an elimination process that recognizes wrong bases after replication.

1. How is the DNA polymerase able to select a correct base? Two hypotheses are proposed. The 1st states³⁹⁰ that the strength of the hydrogen bonding between the complementary bases (A–T and G–C) is stronger than other pairing possibilities^{222,392–396} and this so-called passive DNA polymerase model predicts that the most stable base pairs are favored for incorporation while the more weakly ones are rejected before incorporation or, if they are by any chance incorporated, immediately removed by the 3'–5' exonuclease¹⁴². The 2nd^{5,397,398}, the so-called active DNA polymerase model, proposes that hydrogen bonding alone cannot fully explain the accuracy, and postulates allosteric sites on the DNA polymerase that react to the nucleotides in connection with base pairing.

2. An editing mechanism has been detected in DNA polymerase I¹⁴². The polymerase polypeptide possesses an additional enzyme activity, the 3'–5' exonuclease. This activity is able to remove incorrect bases as soon as they are incorporated. Such a 'proofreading' exonuclease was later found in T4¹³⁸, T5³⁹⁹ and T7¹³⁷, as well as in the *Escherichia coli* replicase, DNA polymerase III⁵³. It was detected in lower eukaryotes¹⁴⁵ and even in mammalian DNA polymerase^{44,91}. This enzyme, DNA polymerase δ , has been purified to apparent homogeneity¹⁰¹ and appears to have this associated exonuclease in the DNA polymerase polypeptide^{91,101}. As mentioned earlier, it cannot be ruled out that the δ polymerase is a form of DNA polymerase α and this has indeed been proposed⁹¹.

Genetic studies have given insight into this editing mechanism. Mutants of T4 with mutator and antimu-

tator phenotype, mutated in gene 43, the genetic locus for the DNA polymerase, seem to have either an altered base-selection mode^{400,401} or an altered ratio of DNA polymerase to 3'–5' exonuclease activity^{392,395}.

3. The postulation of the third step, editing after replication, has led to the discovery of enzymes that can recognise and eliminate incorrect bases. Among these are DNA glycosylases and apurinic endonucleases⁴⁰² and also an 'insertase', an enzyme capable of purine insertion at apurinic sites in double-stranded DNA⁴⁰³. Newly replicated DNA might be undermethylated⁴⁰⁴, allowing mother and daughter strands to be discriminated and a mechanism has been described that scans the newly replicated DNA for mismatched base pairs and excises the incorrectly incorporated nucleotide on the daughter strand⁴⁰⁵.

Several in vitro fidelity assays have been developed, and give some insight into the correct copying ability of different DNA polymerases. The first used artificial polynucleotide template such as homopolymers and heteropolymers^{172,195,407}. The incorporation of a correct versus an incorrect nucleotide gives an estimate for misincorporation. Results strongly depend on the extremely high purity of the template and the nucleotides. Using such artificial templates, the following estimated misincorporations were obtained: DNA polymerase I, 1.3×10^{-5} ^{172,173}, T4 DNA polymerase, 10^{-5} ⁴⁰⁷, vertebrate DNA polymerases α ^{195,196} from sea urchin, 8×10^{-5} and calf thymus, 1.1×10^{-4} , vertebrate DNA polymerases β ^{195,196} from human placenta, 2.5×10^{-5} and calf thymus, 3.3×10^{-5} . The accuracy of a variety of other DNA polymerases have been determined^{5,196,408–410} and sometimes conflicting results have been reported. These results have to be seen in the context of differences in the degree of purity of templates and substrates and the various forms of enzymes that were used. Additional proteins, or different divalent cations and other factors are sometimes used by different authors. Using these accuracy measurement methods it seems that the β enzyme (repair) is more faithful than the α -enzyme (replication)^{195,196,411}.

Better in vivo systems were developed by using Φ X174 DNA with an amber mutation (*am3*)⁴¹². A restriction fragment is hybridized just ahead of the mutation. After the DNA polymerase reaction has taken place, the DNA is isolated, *Escherichia coli* spheroblasts are infected and on indicator bacteria the reversion frequencies are determined. With this system the accuracy of DNA polymerase I was 1.5×10^{-6} , an order of magnitude higher than previously determined¹⁷⁴. On the other hand, highly purified α -polymerases had an accuracy of 3×10^{-5} and the β and γ enzymes of 2×10^{-4} and 1.4×10^{-4} , respectively⁴¹³. This suggests that the highly purified enzymes lack factors that are involved in accurate replication. Using randomly nicked double-stranded Φ X174

DNA again with an amber mutation the T4 replication apparatus can replicate such a molecule in the 'rolling circle' mode³²⁹. After isolation and specific restriction and ligation of the DNA, a transfection is performed as mentioned above. Error rates of 2×10^{-6} – 2×10^{-7} were determined with this intact replication apparatus^{329,414}. Finally, the system closest to the situation in vivo is the double-stranded to single-stranded Φ X174 DNA replication³⁷⁰. Using *geneA* protein, *rep* protein, SSB and DNA polymerase III holoenzyme, 10–20 intact viral single-stranded DNA copies can be generated³⁷⁰. Applying the mentioned transfection protocol and extrapolation to physiological precursor nucleotide concentrations in *E. coli* an error rate of less than 5×10^{-7} was calculated¹⁸², very similar to that predicted from genetic studies with Φ X174¹⁸⁴. Using this type of in vivo system measurements of mismatches of certain base pair combinations have validated theoretical in vitro models⁴¹⁵.

Both the *E. coli* and the T4 systems give an error rate close to the in vivo rate, in phages. It can therefore be inferred that base selection and proof-reading alone contribute to a high degree of fidelity. However, there is still a factor of 1000 missing compared to the in vivo rate in *E. coli*. A recently described post-replication repair system increases the accuracy by a factor of 10^3 – 10^4 ⁴⁰⁵. Therefore the in vitro determined fidelity of 10^{-5} – 10^{-6} is sufficient to account for a DNA elongation machinery like that in vivo^{182,329}.

From this discussion it seems that the additional proteins in the elongation machinery significantly enhance accuracy. This is evident from comparisons of values between the in vivo-like systems of T4 and *E. coli* versus isolated pure or partially pure enzymes. Such proteins may help to transfer and to complex the replicase to the right primer terminus³²², proof-read the DNA, or make the DNA polymerase more processive^{183,331}. *E. coli* single-stranded DNA binding protein increased the fidelity of prokaryotic and eukaryotic DNA polymerases, probably by enhancing the base selection by the enzyme⁴¹⁶.

A new protocol for testing the error rate of chromatin-associated DNA polymerase β has been published⁴¹⁷. With this method the accuracy is different from previously determined values^{195,413}, probably due to factors associated with the chromatin-DNA polymerase complex. Finally, age-dependent changes in fidelity have been detected in some cases^{408,418}, but not in others^{419–421}.

This could reflect another case of the presence or absence of fidelity factors in different DNA polymerase preparations (see e.g. Murray⁴²²). Therefore increased errors may be due to alteration in factors rather than to the DNA polymerase itself.

In conclusion, the DNA polymerase peptide alone seems to contribute substantially to the correctness of

replication. Studies with in vivo-like prokaryotic systems predict that additional 'accuracy helper proteins' are present.

e) Comparative summary

The action of DNA polymerases and their associated proteins at the replication fork is extremely complex. In prokaryotes, systems elongating in vitro primed DNAs with in vivo speed and accuracy have been designed. In comparison, knowledge of DNA elongation in eukaryotes is still very fragmentary. The use of well-defined prokaryotic template systems and improved knowledge of the genetics of eukaryotes may help to overcome this problem.

5. Proteolysis: an undesirable artefact or an important physiological event?

Many proteins and enzymes have to be cleaved proteolytically before they are able to perform in vivo functions³⁹¹. The molecules may thus be activated (e.g. hormones or other secretory proteins)⁴²³ or fit into a higher order structure (functional complex)⁴²⁴. Proteolytic inactivation of a protein is another example of regulation (e.g. inactivation of *E. coli* bacteriophage lambda repressor by proteolytic cleavage mediated by *recA* protein)⁴²⁵.

Proteolysis can either be genuine (genetically controlled) or artificial (uncontrolled during fractionation and isolation). Uncontrolled proteolysis is probably responsible for a lot of the controversy in the field of eukaryotic DNA polymerases. The use of efficient protease inhibitors should soon give more consistent results. It has been demonstrated that the popular phenylmethylsulfonyl fluoride is inefficient in preventing the formation of low-molecular-weight DNA polymerases¹²⁵. Indeed, the DNA polymerase α from *Drosophila melanogaster* with a high-molecular-weight catalytic subunit can only be isolated in the presence of more suitable inhibitors^{25,75}. In their absence a great variety of different forms is found⁷⁴. Generally it can be stated that the molecular weights of DNA polymerases are found to be higher in the presence of protease inhibitors (table 5).

These facts do not, however, exclude the possibility that genetically programmed conversion of a DNA polymerase precursor by proteolytic cleavage may regulate DNA replication in advance of initiation and elongation. Precursor and product may have different affinities for the primers^{426,427} or for the primosome³²⁴.

In a proteolysis model, as presented in figure 1, a precursor DNA polymerase is cleaved so that the product DNA polymerase can form a holoenzyme complex with auxiliary proteins (subunits). This inactive holoenzyme is further cleaved, so that in-

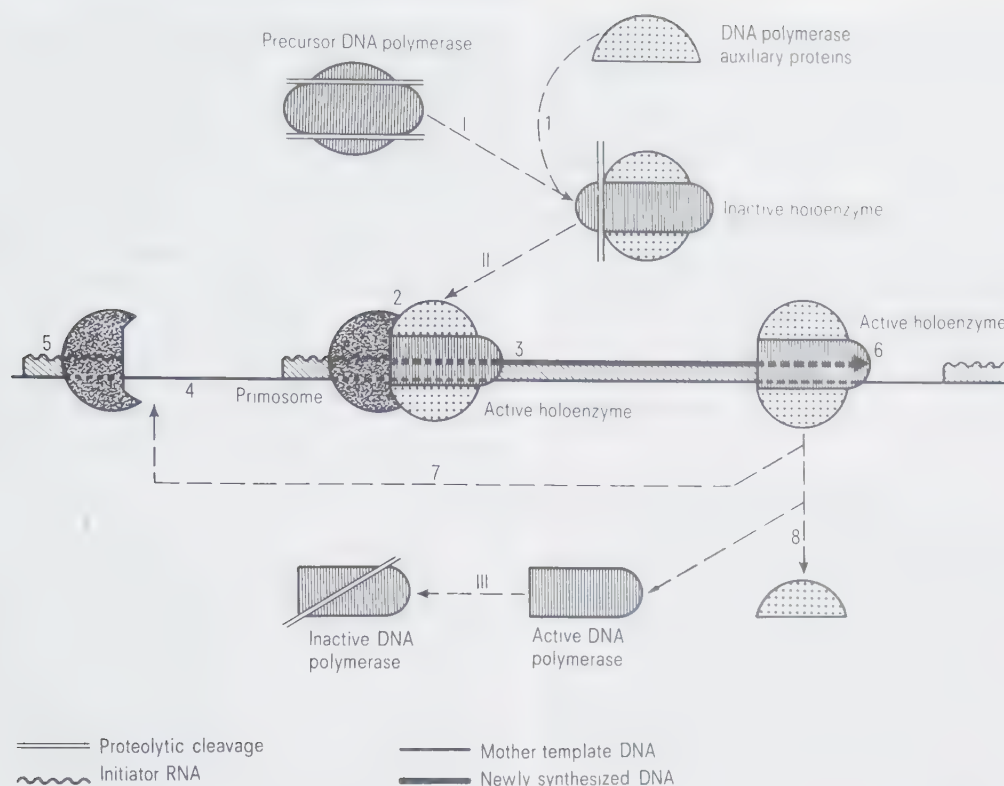


Figure 1. Model postulating proteolytic regulation of a replicative DNA polymerase. Three proteolytic steps (I-III) are proposed for the activation and inactivation of a DNA elongation mechanism. I A precursor DNA polymerase is cleaved so that the DNA polymerase can form a holoenzyme-complex with DNA polymerase auxiliary proteins (1). II This inactive holoenzyme is further cleaved so that interaction with the primosome at the primer terminus is possible (2). After complex formation the DNA polymerase starts DNA synthesis (3) and the primosome moves in anti-elongation direction (4) to form another primer (5) and to wait for another active holoenzyme. When replication is completed (6) (e.g. the previous primer is reached), the active holoenzyme either moves or jumps in the anti-elongation direction to the next primosome (7) or dissociates (8) into the core enzyme and the auxiliary proteins, III The core DNA polymerase is eventually cleaved and inactivated.

interaction with the primosome at the primer/terminus is possible. After complex formation the DNA polymerase starts to synthesize DNA and the primosome moves in the antielongation direction to form another primer and to wait for another holoenzyme. When replication is completed (e.g. the previous primer is reached), the active holoenzyme either moves or jumps in the antielongation direction to the next primosome or dissociates into the core enzyme and the auxiliary proteins. The core DNA polymerase is eventually cleaved and inactivated.

Fragmentary data suggest such a model. *Escherichia coli* DNA polymerase I can be proteolytically split into a lower molecular weight form^{428,429}. The larger fragment (Klenow fragment) is the DNA polymerase with the associated 3'-5' exonuclease⁴³⁰, while the smaller has the 5'-3' exonuclease activity⁴³¹. In eukaryotes it was reported that high-molecular-weight DNA polymerase forms (DNA polymerases α ?) can be converted via intermediate forms into small-molecular-weight ones⁴³²⁻⁴³⁴. In calf thymus extensive studies with DNA polymerase α suggested that proteolysis might be responsible for some of the 5 different forms (A₁, A₂, B, C and D) described^{90,339,435}. It

must be pointed out again, that the different types of DNA polymerase α reported are possibly fragmentary forms of a holoenzyme. 1 or 2 of these calf thymus forms were believed to be triggered by proteolysis³³⁹; this was based on the observation that the activity levels of the different forms have been found to vary in an unpredictable way from preparation to preparation⁴³⁶. Finally a protease from calf thymus was detected that can convert a larger DNA polymerase α (7.4S) to a smaller form (5.4S)⁴³⁷.

Work with DNA polymerase β is much less controversial and the reported properties and sizes are very similar. Since the enzyme is a repair DNA polymerase^{158,207} and constantly needed in the cell, without great fluctuations, it is not astonishing that it is relatively insensitive to proteolytic regulation.

In *Drosophila melanogaster* an active intermediate molecular weight 5.5S DNA polymerase α form was created from a 7.3S precursor by in vitro trypsin treatment^{74,438}. Exposure of HeLa cells to the protein synthesis inhibitor cycloheximide creates a new form of DNA polymerase α ⁴³⁹ and this could be due to the action of a protease released in the cell during such treatment⁴³⁹. On SDS-polyacrylamide gels, after

renaturation of DNA polymerase α ¹²⁴, at least 7 DNA polymerase activities can be detected when limited proteolysis is allowed to occur¹²⁵. The formation of the lower molecular weight fragments can be prevented by more than 90% in the presence of potent protease inhibitors¹²⁵.

In conclusion, these results suggest that not all the proteolytic events detected or triggered in vitro are artefacts. Genetically programmed proteolysis may be an important step in controlling the active participation of a replicase in a replication complex.

6. Evolutionary aspects

Vital functions such as DNA replication and DNA repair are thought to be conserved throughout biological evolution¹. The role of a DNA polymerase, i.e. catalysis of DNA elongation, appears to be similar in all organisms investigated and this enzyme might be expected to be highly conserved. Indeed, all DNA polymerases so far described need a 3'-hydroxyl group of a small RNA or DNA fragment hybridized to a DNA template in order to start polymerization. The direction of polymerization is always 5'-3' in the newly synthesized strand.

Phylogenetic studies have given insight into the presence of different DNA polymerases in species ranging from bacteria to mammals^{162, 440-442} and immunological approaches have shown relatedness between different organisms¹¹⁶. These studies can be summarized as follows: A DNA polymerase α -like enzyme can be found in mammals, birds, reptiles, amphibians, echi-

noids, cephalopods, hydrozoas, sponges, ciliates, flagellates and even in plants and fungi⁴⁴¹. A β -like enzyme seems to be present in all multicellular organisms other than plants and fungi⁴⁴¹. The structural homology between DNA polymerases β from several mammals (mouse, rat, rabbit, pig, calf) and birds has been established^{86, 155, 443}. Finally the γ -like polymerase is present in all mitochondria tested⁴⁴² and also occurs in chloroplasts from plants⁴⁴⁴.

Since the major replicase, the DNA polymerase α appears even in plants and fungi its degree of conservation is higher than that of the β and γ enzymes. Limited proteolysis of crude enzyme fractions from *Escherichia coli* mutants (*polA*, *polB*), *Ustilago maydis*, *Drosophila melanogaster*, rat, calf and human followed by analysis of the activity patterns after SDS-gel electrophoresis and renaturation of the enzymes^{124, 338} gives fragments which show a remarkable similarity in size and enzymatic activity¹²⁵. Even the major *Escherichia coli* replicase, the DNA polymerase III, has at least 3-4 proteolytic fragments in common with the vertebrate and mammalian DNA polymerases¹²⁵ (fig. 2). These similarities may result from the conservation of polypeptide conformations which are particularly susceptible to proteolytic cleavage. Whether only the three-dimensional structure of the replicases or even the primary one is conserved cannot be answered at this stage. It seems, however, that the basic reaction mechanism of incorporating a complementary nucleoside monophosphate has exerted an immense constraint on the overall three-dimensional shape of this biologically most important enzyme.

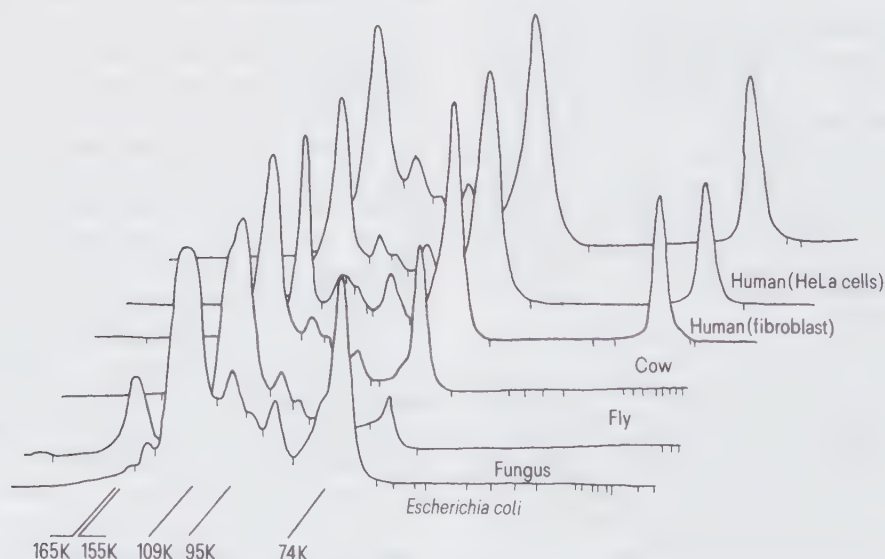


Figure 2. Densitograms of autoradiograms of DNA polymerase activities after SDS/7.5% polyacrylamide gel electrophoresis of different prokaryotic and eukaryotic cell extracts. Storage of crude enzyme fractions to trigger the generation of active proteolytic fragments, electrophoresis of 200 μ g crude enzyme fraction of each tissue, renaturation of DNA polymerase activities in the gel and activity measurement were performed as described¹²⁵. The cathode was on the left and the anode on the right, respectively. Molecular weight markers were *Escherichia coli* RNA polymerase β' , β and σ subunits (165, 155 and 95 kdaltons (K), respectively) and *Escherichia coli* DNA polymerase I (109 K) and its Klenow fragment (74 K). Note that a defined and remarkably similar pattern of intermediate molecular weight activities is generated in extracts from prokaryotic, lower and higher eukaryotic cells.

In the same context it is worth mentioning that aphidicolin, a potent inhibitor of DNA replication and DNA polymerase α (see earlier), inhibits α -like polymerases from the whole range of eukaryotes^{291,292}. Since aphidicolin is a competitive inhibitor of dCTP^{445,446} one can argue that even a functional binding site of eukaryotic replicases has been conserved over several hundred million years. To conclude, it appears that the DNA polymerase which is responsible for chromosomal DNA replication has resisted pressure of mutation so efficiently that biochemical, physical and, to some lesser extent, immunological properties have been conserved for more than a billion years.

7. Conclusions and perspectives

Although DNA replication requires the concerted action of many proteins in a complex, the DNA polymerase plays a central role. The function of a DNA polymerase in a particular DNA synthesis process (e.g. DNA replication or DNA repair) is similar across the whole evolutionary spectrum and the basic reaction mechanism is the same for all DNA polymerases investigated.

The most detailed information available stems from DNA polymerases involved in DNA replication. Replicases not only from prokaryotes but recently also from eukaryotes can be obtained in pure form, and more accurate experiments may now be performed. Prokaryotes, due to the obvious advantages of their genetics, fast generation time and quantity, can teach us in astonishing detail how the mechanism of DNA elongation works. These processes include the interaction of the replicase (DNA polymerase holoenzyme) at the primer/terminus, the mode of translocation after condensation of a nucleoside triphosphate, the degree of accuracy and the concerted action of DNA synthesis and unwinding of the double helix. Even though information from eukaryotes is still fragmentary, comparisons between prokaryotes and eukaryotes may be permitted at this stage:

a) Several DNA polymerases have been identified in one cell and these enzymes have different *in vivo* functions.

b) One DNA transaction (e.g. DNA replication or DNA repair) seems to use more than one DNA polymerase.

c) The major replicase of prokaryotes and eukaryotes works at the replication fork as a multipolypeptide complex, also called DNA polymerase holoenzyme. The additional subunits, also called accessory proteins, may be important for processivity, accuracy and interaction with other proteins (e.g. primosome, unwinding proteins, single-stranded DNA binding proteins).

d) Proteolysis may be important as a regulatory step in prokaryotes as well as in eukaryotes.

e) Replicases are the most conserved DNA polymerases. Base selection as an absolute requirement for a DNA polymerase could have provided a high degree of conservation. This conservation is retained during evolution in the following order: base selection > editing > mode of translocation > accuracy > interaction with proteins at the replication fork. This means that the catalytic DNA polymerase polypeptide is more conserved than the polymerase holoenzyme and the latter more than the entire primosome or replisome.

Future studies with prokaryotes will lead to an understanding of the detailed mechanism of the concerted action between the core DNA polymerase and the associated subunits or auxiliary proteins. In eukaryotes DNA polymerase subunits will be identified and their interaction in the polymerase worked out. In 10 years time most details of the prokaryotic DNA elongation machinery will probably have been elaborated, while for eukaryotes we will have reached roughly the stage we have now reached in our understanding of prokaryotes.

New techniques such as genetic engineering, the use of monoclonal antibodies, and the study of eukaryotic genetics in cell cultures not depending on fetal calf serum, will hopefully help to narrow the gap that exists in the knowledge of prokaryotic and eukaryotic DNA enzymology.

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- 1 Kornberg, A., DNA Replication. W.H. Freeman and Company, San Francisco 1980.
- 2 Kornberg, A., Lehman, I.R., Bessman, M.J., and Simms, E.S., *Biochim. biophys. Acta* 21 (1956) 197-198.
- 3 Tait, R.C., and Smith, D.W., *Nature* 249 (1974) 116-119.
- 4 Kornberg, T., and Kornberg, A., (1974) in: *The Enzymes*, vol. 10, pp. 119-144. Ed. P.D. Boyer. Academic Press, New York 1974.

- 5 Loeb, L.A., in: *The Enzymes*, vol. 10, pp. 173-209. Ed. P.D. Boyer. Academic Press, New York 1974.
- 6 Bollum, F.J., *Prog. nucl. Acid Res. molec. Biol.* 15 (1975) 109-144.
- 7 Weissbach, A., *Cell* 5 (1975) 101-108.
- 8 Holmes, A.M., and Johnston, I.R., *FEBS Lett.* 60 (1975) 233-243.
- 9 Wintersberger, E., *Trends biol. Sci.* 2 (1977) 58-61.
- 10 Weissbach, A., *A. Rev. Biochem.* 46 (1977) 25-47.
- 11 Falaschi, A., and Spadari, S., in: *DNA Synthesis: Present and Future*, pp. 487-515. Eds I. Molineux and M. Kohiyama. Plenum Press, New York 1978.
- 12 Sarngadharan, M.G., Robert-Guroff, M., and Gallo, R.C., *Biochim. biophys. Acta* 516 (1978) 419-487.
- 13 Sheihin, R., Humbert, J., and Pearlman, R.E., *A. Rev. Biochem.* 47 (1978) 277-316.

- 14 Weissbach, A., *Archs Biochem. Biophys.* 198 (1979) 386-396.
- 15 De Pamphilis, M.L., and Wassarman, P.M., *A. Rev. Biochem.* 49 (1980) 627-666.
- 16 Weissbach, A., Baltimore, D., Bollum, F.J., Gallo, R.C., and Korn, D., *Science* 191 (1975) 1183-1185.
- 17 Jeggo, P.A., and Banks, G.R., *Molec. gen. Genet.* 142 (1975) 209-224.
- 18 Sugino, A., and Kakayama, K., *Proc. natl Acad. Sci. USA* 77 (1980) 7049-7053.
- 19 Low, R.K., Arai, K.-I., and Kornberg, A., *Proc. natl Acad. Sci. USA* 78 (1981) 1436-1440.
- 20 Hanawalt, P.C., Cooper, P.K., Ganesan, A.K., and Smith, C.A., *A. Rev. Biochem.* 48 (1979) 783-836.
- 21 Radding, C.M., *A. Rev. Biochem.* 47 (1978) 847-880.
- 22 McHenry, C., and Kornberg, A., *J. biol. Chem.* 252 (1977) 6478-6484.
- 23 Wickner, S., *Proc. natl Acad. Sci. USA* 73 (1976) 3511-3515.
- 24 Liu, C.C., Burke, R.L., Hibner, U., Barry, J., and Alberts, B., *Cold Spring Harb. Symp. quant. Biol.* 43 (1979) 469-487.
- 25 Villani, G., Fay, P.J., Bambara, R.A., and Lehman, I.R., *J. biol. Chem.* 256 (1981) 8202-8207.
- 26 Kollek, R., and Goulian, M., *Proc. natl Acad. Sci. USA* 78 (1981) 6206-6210.
- 27 Reichard, P., and Eliasson, R., *Cold Spring Harb. Symp. quant. Biol.* 43 (1979) 271-277.
- 28 Tsubota, Y., Waqar, M.A., Burke, J.F., Milavetz, B.I., Evans, M.J., Kowalski, D., and Huberman, J.A., *Cold Spring Harb. Symp. quant. Biol.* 43 (1979) 693-704.
- 29 De Pamphilis, M.L., Anderson, S., Bar-Shavit, R., Collins, E., Edenberg, H., Herman, T., Karas, B., Kaufman, G., Krokan, H., Shelton, E., Su, R., Tapper, D., and Wassarman, P.M., *Cold Spring Harb. Symp. quant. Biol.* 43 (1979) 679-692.
- 30 Challberg, M.D., and Kelly, T., *Proc. natl Acad. Sci. USA* 76 (1979) 655-659.
- 31 Drake, J.W., *Nature* 221 (1969) 1132.
- 32 Lehman, I.R., Bessman, M.J., Simms, E.S., and Kornberg, A., *J. biol. Chem.* 233 (1958) 163-170.
- 33 Bollum, F.J., and Potter, V.R., *J. Am. chem. Soc.* 79 (1957) 3603-3604.
- 34 Bollum, F.J., and Potter, V.R., *J. biol. Chem.* 233 (1958) 478-482.
- 35 Kornberg, T., and Gefter, M.L., *Biochem. biophys. Res. Commun.* 40 (1970) 1348-1355.
- 36 Moses, R.E., and Richardson, C.C., *Biochem. biophys. Res. Commun.* 41 (1970) 1557-1564.
- 37 Knippers, R., *Nature* 228 (1970) 1050-1053.
- 38 Kornberg, T., and Gefter, M.L., *Proc. natl Acad. Sci. USA* 68 (1971) 761-764.
- 39 Nüsslein, V., Otto, B., Bonhoeffer, F., and Schaller, H., *Nature New Biol.* 234 (1971) 285-286.
- 40 Bollum, F.J., *J. biol. Chem.* 235 (1960) 2399-2403.
- 41 Weissbach, A., Schlabach, A., Fridlender, B., and Bolden, A., *Nature New Biol.* 231 (1971) 167-170.
- 42 Baril, E.F., Brown, O.E., Jenkins, M.D., and Laszlo, J., *Biochemistry* 10 (1971) 1981-1992.
- 43 Fridlender, B., Fry, M., Bolden, A., and Weissbach, A., *Proc. natl Acad. Sci. USA* 69 (1972) 452-455.
- 44 Byrnes, J.J., Downey, K.M., Black, V.L., and So, A.G., *Biochemistry* 15 (1976) 2817-2823.
- 45 Richardson, C.C., Schildkraut, C.L., Aphosian, H.V., and Kornberg, A., *J. biol. Chem.* 239 (1964) 222-232.
- 46 Jovin, T.M., Englund, P.T., and Bertsch, L.L., *J. biol. Chem.* 244 (1969) 2996-3008.
- 47 Wickner, R.B., Ginsburg, B., Berkower, I., and Hurwitz, J., *J. biol. Chem.* 247 (1972) 489-497.
- 48 Wickner, R.B., Ginsburg, B., and Hurwitz, J., *J. biol. Chem.* 247 (1972) 498-504.
- 49 Gefter, M.L., Molineux, I.J., Kornberg, T., and Khorana, H.G., *J. biol. Chem.* 247 (1972) 3321-3326.
- 50 Kornberg, T., and Gefter, M.L., *J. biol. Chem.* 247 (1972) 5369-5375.
- 51 Otto, B., Bonhoeffer, F., and Schaller, H., *Eur. J. Biochem.* 34 (1973) 440-447.
- 52 Livingston, D.M., Hinkle, D.C., and Richardson, C.C., *J. biol. Chem.* 250 (1975) 461-469.
- 53 Livingston D.M., and Richardson, C.C., *J. biol. Chem.* 250 (1975) 470-478.
- 54 McHenry, C.S., and Crow, W., *J. biol. Chem.* 254 (1979) 1748-1753.
- 55 Modrich, P., and Richardson, C.C., *J. biol. Chem.* 250 (1975) 5515-5522.
- 56 Hori, K., Mark, D.F., and Richardson, C.C., *J. biol. Chem.* 254 (1979) 11591-11597.
- 57 Goulian, M., Lucas, Z.J., and Kornberg, A., *J. biol. Chem.* 243 (1969) 627-638.
- 58 Fujimura, R.K., and Ropp, B.C., *J. biol. Chem.* 251 (1976) 2168-2175.
- 59 Yehle, C.O., and Ganesan, A.T., *J. biol. Chem.* 248 (1973) 7456-7463.
- 60 Ganesan, A.T., Yehle, C.O., and Yu, C.C., *Biochem. biophys. Res. Commun.* 50 (1973) 155-163.
- 61 Gass, K.B., and Cozzarelli, N.R., *J. biol. Chem.* 248 (1973) 7688-7700.
- 62 Low, R.L., Rashbaum, S.A., and Cozzarelli, N.R., *J. biol. Chem.* 251 (1976) 1311-1325.
- 63 Harwood, S.J., Scheudel, R.F., and Wells, R.D., *J. biol. Chem.* 245 (1970) 5614-5624.
- 64 Hamilton, L., Mahler, I., and Grossman, L., *Biochemistry* 13 (1974) 1886-1896.
- 65 Engler, M.J., and Bessman, M.J., *Cold Spring Harb. Symp. quant. Biol.* 43 (1979) 929-935.
- 66 Banks, G.R., Holloman, W.K., Kairis, M.V., Spanos, A., and Yarranton, G.T., *Eur. J. Biochem.* 62 (1976) 131-142.
- 67 Wintersberger, E., *Eur. J. Biochem.* 50 (1974) 41-47.
- 68 Chang, L.M.S., *J. biol. Chem.* 252 (1977) 1873-1880.
- 69 McLennan, A.G., and Keir, H.M., *Biochem. J.* 151 (1975) 223-237.
- 70 Baer, A., and Schiebel, W., *Eur. J. Biochem.* 86 (1978) 77-84.
- 71 Tait, A., and Cummings, D.J., *Biochim. biophys. Acta* 378 (1975) 382-385.
- 72 Crerar, M., and Pearlman, R.E., *J. biol. Chem.* 249 (1974) 3123-3131.
- 73 Loomis, L.W., Rosamondo, E.F., and Chang, L.M.S., *Biochim. biophys. Acta* 125 (1976) 469-477.
- 74 Brakel, C.L., and Blumenthal, A.B., *Biochemistry* 16 (1977) 3137-3143.
- 75 Banks, G.R., Boezi, J.A., and Lehman, I.R., *J. biol. Chem.* 254 (1979) 9886-9892.
- 76 Furia, M., Polito, L.C., Locorotondo, E., and Grippo, P., *Nucl. Acids Res.* 6 (1979) 3399-3410.
- 77 Racine, F.M., and Morris, P.W., *Nucl. Acids Res.* 5 (1978) 3945-3957.
- 78 Habara, A., Nagano, H., and Mano, Y., *Biochim. biophys. Acta* 561 (1979) 17-28.
- 79 Morris, P.W., and Rutter, W.J., *Biochemistry* 15 (1976) 3106-3113.
- 80 De Petrocellis, B., Parisi, E., Filosa, S., and Capasso, A., *Biochem. biophys. Res. Commun.* 68 (1976) 954-960.
- 81 Tato, F., Attardi-Gandini, D., and Tocchini-Valentini, G.P., *Proc. natl Acad. Sci. USA* 71 (1974) 3706-3710.
- 82 Grippo, P., Locorotondo, G., and Caruso, A., *FEBS Lett.* 51 (1975) 137-142.
- 83 Benbow, R.M., Breaux, C.B., Joenje, H., Krauss, M.R., Lennox, R.W., Nelson, E.M., Wang, N.S., and White, S.H., *Cold Spring Harb. Symp. quant. Biol.* 43 (1979) 597-602.
- 84 Joenje, H., and Benbow, R.M., *J. biol. Chem.* 253 (1978) 2640-2649.
- 85 Brun, G., Rougeon, F., Lauber, M., and Chapeville, F., *Eur. J. Biochem.* 41 (1974) 241-251.
- 86 Yamaguchi, M., Tanabe, K., Taguchi, Y.N., Nishizawa, M., Takahashi, T., and Matsukage, A., *J. biol. Chem.* 255 (1980) 9942-9948.
- 87 Yamaguchi, M., Matsukage, A., and Takahashi, T., *Nature* 285 (1980) 45-47.
- 88 Yamaguchi, M., Matsukage, A., and Takahashi, T., *J. biol. Chem.* 255 (1980) 7002-7009.
- 89 Fisher, P.A., and Korn, D., *J. biol. Chem.* 252 (1977) 6528-6535.
- 90 Holmes, A.M., Hesselwood, I.P., and Johnston, I.R., *Eur. J. Biochem.* 62 (1976) 229-235.
- 91 Chen, Y.C., Bohn, E.W., Planck, S.R., and Wilson, S.H., *J. biol. Chem.* 254 (1979) 11678-11687.
- 92 Grummt, F., Waltl, G., Jantzen, J., Hamprecht, K., Hübscher, U., and Kuenzle, C.C., *Proc. natl Acad. Sci. USA* 76 (1979) 6081-6085.

- 93 Méchali, M., Abadiebat, J., and de Recondo, A.M., *J. biol. Chem.* 255 (1980) 2114-2122.
- 94 Grosse, F., and Krauss, G., *Nucl. Acids Res.* 8 (1980) 5703-5714.
- 95 Grosse, F., and Krauss, G., *Biochemistry* 20 (1981) 5470-5475.
- 96 Chang, L.M.S., *J. biol. Chem.* 248 (1973) 3789-3795.
- 97 Wang, T.S.-F., Sedwick, W.D., and Korn, D., *J. biol. Chem.* 249 (1974) 841-850.
- 98 Spadari, S., and Weissbach, A., *J. biol. Chem.* 249 (1974) 5809-5815.
- 99 Knopf, K.W., Yamada, M., and Weissbach, A., *Biochemistry* 15 (1976) 4540-4548.
- 100 Hübscher, U., Kuenzle, C.C., and Spadari, S., *Eur. J. Biochem.* 81 (1977) 249-258.
- 101 Lee, M.Y.W.F., Tan, C.-K., So, A.G., and Downey, K.M., *Biochemistry* 19 (1980) 2096-2101.
- 102 Hübscher, U., and Kornberg, A., *Proc. natl Acad. Sci. USA* 76 (1979) 6284-6288.
- 103 Johanson, K.O., and McHenry, C.S., *J. biol. Chem.* 255 (1980) 10984-10990.
- 104 Hübscher, U., and Kornberg, A., *J. biol. Chem.* 255 (1980) 11698-11703.
- 105 Burgers, P.M.J., Kornberg, A., and Sakakibara, Y., *Proc. natl Acad. Sci. USA* 78 (1981) 5391-5395.
- 106 Arai, K.-I., and Kornberg, A., *Proc. natl Acad. Sci. USA* 78 (1981) 69-73.
- 107 Arai, K.-I., Low, R.L., and Kornberg, A., *Proc. natl Acad. Sci. USA* 78 (1981) 707-711.
- 108 Shlomai, J., Polder, L., Arai, K.-I., and Kornberg, A., *J. biol. Chem.* 256 (1981) 5233-5238.
- 109 Arai, N., Polder, L., Arai, K.-I., and Kornberg, A., *J. biol. Chem.* 256 (1981) 5239-5246.
- 110 Fuller, R.S., Kaguni, J.M., and Kornberg, A., *Proc. natl Acad. Sci. USA* 78 (1981) 7370-7374.
- 111 Spadari, S., Muller, R., and Weissbach, A., *J. biol. Chem.* 249 (1974) 2991-2992.
- 112 Brun, G.M., Scovassi, A.I., and Bertazzoni, U., in: *DNA Synthesis: Present and Future*, pp.597-603. Eds I. Molineux and M. Kohiyama. Plenum Press, New York 1978.
- 113 Brun, G.M., Assairi, L.M., and Chapeville, F., *J. biol. Chem.* 250 (1975) 7320-7323.
- 114 Smith, R.G., Abrell, J.W., Lewis, B.J., and Gallo, R.C., *J. biol. Chem.* 250 (1975) 1702-1709.
- 115 Robert-Guroff, M., and Gallo, R.C., *Biochemistry* 16 (1977) 2874-2880.
- 116 Chang, L.M.S., and Bollum, F.J., *J. biol. Chem.* 256 (1981) 494-498.
- 117 Rama Reddy, G.V., Goulian, M., and Hendler, S.S., *Nature New Biol.* 234 (1971) 286-288.
- 118 Edenberg, H.J., Anderson, S., and de Pamphilis, M.L., *J. biol. Chem.* 253 (1978) 3273-3280.
- 119 Ikegami, S., Tagushi, T., Ohashi, M., Oguro, M., Najano, H., and Mano, Y., *Nature* 275 (1978) 458-460.
- 120 Sabourin, C.L.K., Reno, J.M., and Boezi, J.A., *Archs Biochem. Biophys.* 187 (1978) 96-101.
- 121 Kornberg, A., *Cold Spring Harb. Symp. quant. Biol.* 43 (1979) 1-8.
- 122 Welch, M.M., and McHenry, C.S., *Fedn Proc.* 40 (1981) 1903.
- 123 Villani, G., Sauer, B., and Lehman, I.R., *J. biol. Chem.* 255 (1980) 9479-9483.
- 124 Spanos, A., Sedgwick, S.G., Yarranton, G.T., Hübscher, U., and Banks, G.R., *Nucl. Acids Res.* 9 (1981) 1825-1839.
- 125 Hübscher, U., Spanos, A., Albert, W., Grummt, F., and Banks, G.R., *Proc. natl Acad. Sci. USA* 78 (1981) 6771-6775.
- 126 Spadari, S., and Weissbach, A., *J. molec. Biol.* 86 (1974) 11-20.
- 127 Chiu, R.W., and Baril, E.F., *J. biol. Chem.* 250 (1975) 7951-7957.
- 128 Hübscher, U., Kuenzle, C.C., and Spadari, S., *Nucl. Acids Res.* 4 (1977) 2917-2929.
- 129 Hossli, R., Thesis, University of Zürich, Zürich 1976, Switzerland.
- 130 Hübscher, U., Thesis, University of Zürich, Zürich 1976, Switzerland.
- 131 Baril, E.F., Jenkins, M.D., Brown, D.E., Lazlo, J., and Morris, H.P., *Cancer Res.* 33 (1973) 1187-1193.
- 132 William, L.E., Surrey, S., and Lieberman, I., *J. biol. Chem.* 250 (1975) 8179-8183.
- 133 Coleman, M.S., Hutton, I.J., and Bollum, F.J., *Nature* 248 (1974) 407-409.
- 134 Mayer, R.J., Smith, G.R., and Gallo, R.C., *Blood* 46 (1975) 509-518.
- 135 Bertazzoni, U., Stefanini, M., Pedrali Noy, G., Guilotto, E., Nuzzo, F., Falaschi, A., and Spadari, S., *Proc. natl Acad. Sci. USA* 73 (1976) 785-789.
- 136 Nordström, B., Randahl, H., Slaby, I., and Holmgren, A., *J. biol. Chem.* 256 (1981) 3112-3117.
- 137 Hori, K., Mark, D.F., and Richardson, C.C., *J. biol. Chem.* 254 (1979) 11598-11604.
- 138 Lehman, I.R., in: *Methods in Enzymology*, vol.29E, pp.46-53. Eds S.P. Colowick and N.O. Kaplan. Academic Press, New York 1974.
- 139 Stenart, C.D., Anaud, S.R., and Bessman, M.J., *J. biol. Chem.* 243 (1968) 5308-5318.
- 140 Mills, L.B., Stanbridge, E.J., Sedwick, W.D., and Korn, D., *J. Bact.* 132 (1977) 641-649.
- 141 Boxer, L.M., and Korn, D., *Biochemistry* 18 (1979) 4742-4749.
- 142 Brutlag, D., and Kornberg, A., *J. biol. Chem.* 247 (1972) 241-248.
- 143 Cozzarelli, N.R., Kelly, R.B., and Kornberg, A., *J. molec. Biol.* 45 (1969) 513-531.
- 144 Hübscher, U., and Kornberg, A., *J. supramolec. Struct., suppl.* 4 (1980) 368.
- 145 Banks, G.R., and Yarranton, G.T., *Eur. J. Biochem.* 62 (1976) 143-150.
- 146 Yarranton, G.T., and Banks, G.R., *Eur. J. Biochem.* 77 (1977) 521-527.
- 147 Choudry, M.K., and Cox, R.A., in: *Current Research on Physarum*, vol.120, pp.71-76. Ed. W. Sachsenmaier. Publ. University Innsbruck, 1979.
- 148 Schiebel, W., and Raffael, A., *FEBS Lett.* 121 (1980) 81-85.
- 149 Furukawa, Y., Yamada, R., and Kohno, M., *Nucl. Acids Res.* 7 (1979) 2387-2398.
- 150 Baril, E.F., Scheiner, C., and Pederson, T., *Proc. natl Acad. Sci. USA* 77 (1980) 3317-3321.
- 151 Loeb, L.A., *J. biol. Chem.* 244 (1969) 1672-1681.
- 152 Hobart, P.M., and Infante, A.A., *J. biol. Chem.* 253 (1978) 8229-8238.
- 153 Chang, L.M.S., and Bollum, F.J., *J. biol. Chem.* 248 (1973) 3398-3404.
- 154 Lee, M.Y.W.F., Tan, C.-K., Downey, K.M., and So, A.G., *Prog. nucl. Acid Res. molec. Biol.* 26 (1981) 83-96.
- 155 Tanabe, K., Yamaguchi, M., Matsukage, A., and Takahashi, T., *J. biol. Chem.* 256 (1981) 3098-3102.
- 156 Tanabe, K., Bohn, E.W., and Wilson, S.H., *Biochemistry* 18 (1979) 3401-3406.
- 157 Stalker, D.M., Mosbaugh, D.W., and Meyer, R.R., *Biochemistry* 15 (1976) 3114-3121.
- 158 Waser, J., Hübscher, U., Kuenzle, C.C., and Spadari, S., *Eur. J. Biochem.* 97 (1979) 361-368.
- 159 Kunkel, T.A., Tscheng, J.E., and Meyer, R.R., *Biochim. biophys. Acta* 520 (1978) 302-316.
- 160 Bolden, A., Pedrali Noy, G., and Weissbach, A., *J. biol. Chem.* 252 (1977) 3351-3356.
- 161 Lehman, I.R., and Uyemura, D.G., *Science* 193 (1976) 963-969.
- 162 Scovassi, A.I., Plevani, P., and Bertazzoni, U., *Trends biochem. Sci.* 5 (1980) 335-337.
- 163 Fry, M., in: *Enzymes of DNA Synthesis and Processing*. Ed. S.T. Jacob. CRC Press, Cleveland 1982, in press.
- 164 Jovin, T.M., Englund, P.T., and Kornberg, A., *J. biol. Chem.* 244 (1969) 3009-3018.
- 165 Uyemura, D., Bambara, R.A., and Lehman, I.R., *J. biol. Chem.* 250 (1976) 8577-8584.
- 166 Bambara, R.A., Uyemura, D., and Lehman, I.R., *J. biol. Chem.* 251 (1976) 4090-4094.
- 167 Bambara, R.A., Uyemura, D., and Choi, T., *J. biol. Chem.* 253 (1978) 413-423.
- 168 Deutscher, M.P., and Kornberg, A., *J. biol. Chem.* 244 (1969) 3019-3028.
- 169 Deutscher, M.P., and Kornberg, A., *J. biol. Chem.* 244 (1969) 3029-3037.
- 170 Kelly, R.B., Atkinson, M.R., Huberman, J.A., and Kornberg, A., *Nature* 224 (1969) 495-501.

- 171 Sirover, M.A., and Loeb, L.A., *Biochem. biophys. Res. Commun.* **70** (1976) 812-817.
- 172 Trautner, T.A., Swartz, M.N., and Kornberg, A., *Proc. natl Acad. Sci. USA* **48** (1962) 449-455.
- 173 Agarwal, S.S., Diebe, D.K., and Loeb, L.A., *J. biol. Chem.* **254** (1979) 101-106.
- 174 Kunkel, T.A., and Loeb, L.A., *J. biol. Chem.* **255** (1980) 9961-9966.
- 175 Lehman, I.R., and Chien, J.R., *J. biol. Chem.* **248** (1973) 7717-7723.
- 176 Molineux, I.J., and Gefter, M.L., *Proc. natl Acad. Sci. USA* **71** (1974) 3858-3862.
- 177 Molineux, I.J., Friedman, S., and Gefter, M.L., *J. biol. Chem.* **249** (1974) 6090-6098.
- 178 Fujiwara, T., and Komano, T., *Agr. Biol. Chem.* **38** (1974) 1281-1288.
- 179 Molineux, I.J., and Gefter, M.L., *J. molec. Biol.* **98** (1975) 811-825.
- 180 Sherman, L.A., and Gefter, M.L., *J. molec. Biol.* **103** (1976) 61-76.
- 181 Hübscher, U., (1981) unpublished results.
- 182 Fersht, A., *Proc. natl Acad. Sci. USA* **76** (1979) 4946-4950.
- 183 Fay, P.J., Johanson, K.O., McHenry, C.S., and Bambara, R.A., *J. biol. Chem.* **256** (1981) 976-983.
- 184 Korn, D., Eichler, D.C., Fisher, P.A., and Wang, T.S.-F., in: *DNA Synthesis: Present and Future*, pp.517-588. Eds I. Molineux and M. Kohiyama. Plenum Press, New York 1978.
- 185 Korn, D., Fisher, P.A., and Wang, T.S.-F., *Prog. nucl. Acid Res. molec. Biol.* **26** (1981) 63-81.
- 186 Spadari, S., and Weissbach, A., *Proc. natl Acad. Sci. USA* **72** (1975) 503-507.
- 187 Fichot, O., Pascal, M., Méchali, M., and de Recondo, A.-M., *Biochim. biophys. Acta* **561** (1979) 29-41.
- 188 Fisher, P.A., Wang, T.S.-F., and Korn, D., *J. biol. Chem.* **254** (1979) 6128-6137.
- 189 Fisher, P.A., and Korn, D., *J. biol. Chem.* **254** (1981) 11033-11039.
- 190 Fisher, P.A., and Korn, D., *J. biol. Chem.* **254** (1981) 11040-11046.
- 191 Fisher, P.A., Chen, J.T., and Korn, D., *J. biol. Chem.* **256** (1981) 133-141.
- 192 Hockensmith, J.W., and Bambara, P.A., *Biochemistry* **20** (1981) 227-232.
- 193 McKune, K., and Holmes, A.M., *Biochem. biophys. Res. Commun.* **90** (1979) 864-870.
- 194 Das, S.K., and Fujimura, R.K., *J. biol. Chem.* **254** (1979) 1227-1232.
- 195 Loeb, L.A., Weymouth, L.A., Kunkel, T.A., Gopinathan, K.P., Beckman, R.A., and Dube, D.K., *Cold Spring Harb. Symp. quant. Biol.* **43** (1979) 921-935.
- 196 Seal, G., Shearman, C.W., and Loeb, L.A., *J. biol. Chem.* **254** (1979) 5229-5237.
- 197 Wang, T.S.-F., and Korn, D., *Biochemistry* **19** (1980) 1782-1790.
- 198 Waser, J., Thesis, University of Zürich, Zürich 1979, Switzerland.
- 199 Siedlecki, J.A., Szyszko, J., Pietrzykowska, I., and Zmudzka, B., *Nucl. Acids Res.* **8** (1980) 361-375.
- 200 Nowak, R., Zarehska, Z., and Zmudzka, B., *Biochim. biophys. Acta* **609** (1980) 246-256.
- 201 Soltyk, A., Siedlecki, J.A., Pietrzykowska, I., and Zmudzka, B., *FEBS Lett.* **125** (1981) 227-230.
- 202 Matsukage, A., Nishizawa, M., and Takahashi, T., *J. Biochem.* **85** (1979) 1551-1554.
- 203 Matsukage, A., Bohn, E.W., and Wilson, S.H., *Biochemistry* **14** (1975) 1006-1020.
- 204 Robert-Guroff, M., Schrecker, A.W., Brinkman, B.J., and Gallo, R.C., *Biochemistry* **16** (1977) 2866-2873.
- 205 Villani, G., Defais, M., Spadari, S., Caillet-Fauquet, P., Boiteux, S., and Radman, M., in: *Research in Photobiology*, pp.903-922. Ed. A. Castellani. Plenum Press, New York 1977.
- 206 Bertazzoni, U., Scovassi, A.I., and Brun, G.M., *Eur. J. Biochem.* **81** (1977) 237-248.
- 207 Hübscher, U., Kuenzle, C.C., and Spadari, S., *Proc. natl Acad. Sci. USA* **76** (1979) 2316-2320.
- 208 Hübscher, U., Lutz, H., and Kornberg, A., *Proc. natl Acad. Sci. USA* **77** (1980) 5097-5101.
- 209 Hübscher, U., *Experientia* **37** (1981) 649.
- 210 Fisher, P.A., and Korn, D., *Biochemistry* **20** (1981) 4560-4569.
- 211 Fisher, P.A., and Korn, D., *Biochemistry* **20** (1981) 4570-4578.
- 212 Lindahl, T., Gally, J.A., and Edelman, G.M., *Proc. natl Acad. Sci. USA* **62** (1969) 597-603.
- 213 Doniger, J., and Grossman, L., *J. biol. Chem.* **251** (1976) 4579-4587.
- 214 Lindahl, T., Gally, J.A., and Edelman, G.M., *J. biol. Chem.* **244** (1969) 5014-5019.
- 215 Mosbaugh, D.W., and Meyer, R.R., *J. biol. Chem.* **255** (1980) 10239-10247.
- 216 Hollis, G.F., and Grossman, L., *J. biol. Chem.* **256** (1981) 8074-8079.
- 217 Ernster, L., and Schatz, G., *J. Cell Biol.* **91** (1981) 227s-270s.
- 218 Nass, S., and Nass, M.M.K., *J. Cell Biol.* **19** (1963) 613-629.
- 219 Luch, D.J.L., and Reich, E., *Proc. natl Acad. Sci. USA* **52** (1964) 931-938.
- 220 De Luria, P., and Cairns, J., *Nature* **224** (1969) 1164-1166.
- 221 Gefter, M.L., Hirota, Y., Kornberg, T., Wechsler, J.A., and Barnoux, C., *Proc. natl Acad. Sci. USA* **68** (1971) 3150-3153.
- 222 Kornberg, A., *Science* **163** (1969) 1410-1418.
- 223 Kuempel, P.C., and Veomatt, G.E., *Biochem. biophys. Res. Commun.* **41** (1970) 973-980.
- 224 Okazaki, R., Arisawa, M., and Sugino, A., *Proc. natl Acad. Sci. USA* **68** (1971) 2954-2957.
- 225 Okazaki, R., Okazaki, T., Sakabe, K., Sugimoto, K., and Sugino, A., *Proc. natl Acad. Sci. USA* **59** (1968) 598-605.
- 226 Lehman, I.R., *Science* **186** (1974) 790-797.
- 227 Gross, J., and Gross, M., *Nature* **224** (1969) 1166-1168.
- 228 Kato, T., and Kondo, S., *J. Bact.* **104** (1970) 871-881.
- 229 Town, C.D., Smith, K.C., and Kaplan, H.S., *Science* **172** (1971) 851-854.
- 230 Cooper, P.K., and Hanawalt, P.C., *Proc. natl Acad. Sci. USA* **69** (1972) 1156-1160.
- 231 Sedgwick, S.G., and Bridges, B.A., *Nature* **249** (1974) 348-349.
- 232 Niwa, O., Bryan, S.K., and Moses, R.E., *Proc. natl Acad. Sci. USA* **76** (1979) 5572-5576.
- 233 Niwa, O., Bryan, S.K., and Moses, R.E., *Proc. natl Acad. Sci. USA* **78** (1981) 7024-7028.
- 234 Staudenbauer, W.L., *Molec. gen. Genet.* **149** (1976) 151-158.
- 235 Campbell, J.L., Soll, L., and Richardson, C.C., *Proc. natl Acad. Sci. USA* **69** (1972) 2090-2094.
- 236 Tait, R.C., Harris, A.L., and Smith, D.W., *Proc. natl Acad. Sci. USA* **71** (1974) 675-679.
- 237 Masker, W., Hanawalt, P.C., and Shizya, H., *Nature New Biol.* **244** (1973) 242-243.
- 238 Meyer, R.R., Glassberg, J., and Kornberg, A., *Proc. natl Acad. Sci. USA* **76** (1979) 1702-1705.
- 239 Glassberg, J., Meyer, R.R., and Kornberg, A., *J. Bact.* **140** (1979) 14-19.
- 240 Meyer, R.R., Glassberg, J., Scott, J.V., and Kornberg, A., *J. biol. Chem.* **255** (1980) 2897-2901.
- 241 Wickner, W., and Kornberg, A., *J. biol. Chem.* **249** (1974) 6244-6249.
- 242 Youngs, D.A., and Smith, K.C., *Nature New Biol.* **244** (1973) 240-241.
- 243 Ross, S.L., Sharma, S., and Moses, R.E., *Molec. gen. Genet.* **179** (1980) 595-605.
- 244 Tsai, Y.-J., Hanoka, F., Nakano, M.M., and Yamada, M., *Biochem. biophys. Res. Commun.* **91** (1979) 1190-1195.
- 245 Herrick, G., Spear, B.B., and Veomett, G., *Proc. natl Acad. Sci. USA* **73** (1976) 1136-1139.
- 246 Martini, G., Tato, F., Gandini-Attardi, D., and Tocchini-Valentini, G.P., *Biochem. biophys. Res. Commun.* **72** (1976) 875-879.
- 247 Lynch, W.E., Short, J., and Lieberman, J., *Cancer Res.* **36** (1976) 901-904.
- 248 Forster, D.N., and Gurney, T., *J. biol. Chem.* **251** (1976) 7893-7898.
- 249 Brown, M.-K., Bollum, F.J., and Chang, L.M.S., *Proc. natl Acad. Sci. USA* **78** (1981) 3049-3052.
- 250 Chang, L.M.S., and Bollum, F.J., *J. biol. Chem.* **247** (1972) 7948-7950.
- 251 Chang, L.M.S., Brown, M., and Bollum, F.J., *J. molec. Biol.* **74** (1973) 1-8.
- 252 Claycomb, W.C., *J. biol. Chem.* **250** (1975) 3229-3235.

- 253 Hübscher, U., Kuenzle, C.C., Limacher, W., Scherrer, P., and Spadari, S., Cold Spring Harb. Symp. quant. Biol. 43 (1979) 625-629.
- 254 Méchali, M., Girard, M., and de Recondo, A.-M., J. Virol. 23 (1977) 117-125.
- 255 Otto, B., and Fanning, E., Nucl. Acids Res. 5 (1978) 1715-1728.
- 256 Waqar, M.A., Evans, M.J., and Huberman, J.A., Nucl. Acids Res. 5 (1978) 1933-1946.
- 257 Reed, S.I., Ferguson, J., Davies, R.W., and Stark, G.R., Proc. natl Acad. Sci. USA 72 (1975) 1605-1609.
- 258 Arens, M., Yamashita, T., Padmanabhan, R., Tsumo, T., and Green, M., J. biol. Chem. 252 (1977) 7947-7954.
- 259 Brison, O., Keding, C., and Wilhelm, J., J. Virol. 24 (1977) 423-435.
- 260 de Jong, A., van der Vliet, P.Ch., and Jansz, H.S., Biochim. biophys. Acta 467 (1977) 156-165.
- 261 Abboud, M.M., and Horwitz, M.S., Nucl. Acids Res. 6 (1979) 1025-1039.
- 262 Longiaru, M., Ikeda, J.E., Horwitz, S.B., and Horwitz, M.S., Nucl. Acids Res. 6 (1979) 3369-3386.
- 263 Winnacker, E.L., Cell 14 (1978) 761-773.
- 264 Schlaeger, E.-J., van Telgen, H.-J., Klempnauer, K.-H., and Knippers, R., Eur. J. Biochem. 84 (1978) 95-102.
- 265 Pritschard, C., Stout, E.R., and Bates, R.C., J. Virol. 37 (1981) 352-362.
- 266 Kollek, R., Tseng, B.Y., and Goulian, M., J. Virol. 41 (1982) 982-989.
- 267 Zimmermann, W., and Weissbach, A., J. biol. Chem. (1982) submitted.
- 268 Kornberg, A., in: RNA polymerase, pp.331-352. Eds R. Losick and M. Chamberlin. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 1976.
- 269 Otto, B., Baynes, M., and Knippers, R., Eur. J. Biochem. 73 (1977) 17-24.
- 270 Burke, J.F., Plummer, J., Huberman, J.A., and Evans, M.J., Biochim. biophys. Acta 609 (1980) 205-223.
- 271 Ikeda, J.-E., Longiaru, M., Horwitz, M.S., and Hurwitz, J., Proc. natl Acad. Sci. USA 77 (1980) 5827-5831.
- 272 Lamothe, P., Baril, B., Chi, A., Lee, L., and Baril, E., Proc. natl Acad. Sci. USA 78 (1981) 4723-4727.
- 273 Ikeda, J.-E., Enomoto, T., and Hurwitz, J., Proc. natl Acad. Sci. USA (1981) 884-888.
- 274 Ciarocchi, G., Gribock, J., and Linn, S., Nucl. Acids Res. 7 (1979) 1205-1219.
- 275 Hanoka, F., Kato, H., Ikegami, S., Ohashi, M., and Yamada, M., Biochem. biophys. Res. Commun. 87 (1979) 575-580.
- 276 Craddock, V.M., and Ansley, C.M., Biochim. biophys. Acta 564 (1979) 15-22.
- 277 Wawra, E., and Dolejs, I., Nucl. Acids Res. 7 (1979) 675-686.
- 278 Rossignol, J.-M., Gentil, A., Lachapagne, J., and de Recondo, A.-M., Biochem. int. 1 (1980) 253-261.
- 279 Pedrali Noy, G., and Spadari, S., Mutation Res. 70 (1980) 389-394.
- 280 Hübscher, U., Kuenzle, C.C., and Spadari, S., in: DNA Synthesis: Present and Future, pp.605-612. Eds I. Molineux and M. Kohiyama. Plenum Press, New York 1978.
- 281 McLennan, A.G., Biochem. biophys. Res. Commun. 94 (1980) 116-122.
- 282 Zimmermann, W., Chen, S.M., Bolden, A., and Weissbach, A., J. biol. Chem. 255 (1980) 11847-11852.
- 283 Robberson, D.L., Kasamatsu, H., and Vinograd, J., Proc. natl Acad. Sci. USA 69 (1972) 737-741.
- 284 Brown, N.C., and Wright, G.E., Pharmac. Ther. 1 (1977) 437-456.
- 285 Cozzarelli, N.R., A. Rev. Biochem. 46 (1977) 641-648.
- 286 Hübscher, U., (1979) unpublished results.
- 287 Leimbach, S.S., Reno, J.M., Lee, L.F., Isbell, A.F., and Boezi, J.A., Biochemistry 15 (1976) 426-429.
- 288 Yoshida, D., Yamada, M., and Masaki, S., Biochim. biophys. Acta 477 (1977) 144-150.
- 289 Schrecker, A.W., Smith, R.G., and Gallo, R.C., Cancer Res. 34 (1974) 286-292.
- 290 Brunderet, K.M., Dalziel, W., and Hep, P., J. chem. Soc. chem. Commun. (1972) 1027-1028.
- 291 Spadari, S., Trends biochem. Sci. 7 (1982) 29-32.
- 292 Huberman, J.A., Cell 23 (1981) 647-648.
- 293 van der Vliet, P.C., and Kwant, M.M., Nature 276 (1978) 532-534.
- 294 Pedrali Noy, G., and Spadari, S., Biochem. biophys. Res. Commun. 88 (1979) 1194-1202.
- 295 Castellot, J.J., Miller, M.R., Lethomaki, D.M., and Pardee, A.M., J. biol. Chem. 254 (1979) 6904-6908.
- 296 Wist, E., Biochim. biophys. Acta 562 (1979) 62-69.
- 297 Wist, E., and Prudz, H., Nucl. Acids Res. 6 (1979) 1583-1590.
- 298 Krokan, H., Schaffer, P., and de Pamphilis, M.L., Biochemistry 18 (1979) 4431-4443.
- 299 Oguro, M., Shioda, M., Nagano, H., Mano, Y., Hanaoka, F., and Yamada, M., Biochem. biophys. Res. Commun. 92 (1980) 13-19.
- 300 Smith, H.C., and Berezney, R., Biochem. biophys. Res. Commun. 97 (1980) 1541-1547.
- 301 Seki, S., Oda, T., and Ohashi, M., Biochim. biophys. Acta 610 (1980) 413-420.
- 302 Kwant, M.M., and van der Vliet, P.C., Nucl. Acids Res. 8 (1980) 3993-4007.
- 303 Geuskens, M., Hardt, N., Pedrali Noy, G., and Spadari, S., Nucl. Acids Res. 9 (1981) 1599-1613.
- 304 Krokan, H., Wist, E., and Krokan, R.H., Nucl. Acids Res. 9 (1981) 4709-4719.
- 305 Brachet, J., and de Petrocellis, B., Exp. Cell Res. 135 (1981) 179-189.
- 306 Frenkel, G.D., J. Virol. 25 (1978) 459-463.
- 307 van der Vliet, P.C., and Kwant, M.M., Biochemistry 20 (1981) 2628-2632.
- 308 Pincus, S., Robertson, W., and Rekosh, D., Nucl. Acids Res. 9 (1981) 4919-4938.
- 309 Grummt, F., Proc. natl Acad. Sci. USA 75 (1978) 371-375.
- 310 Grummt, F., Cold Spring Harb. Symp. quant. Biol. 43 (1979) 649-653.
- 311 Rapaport, E., Zamecnik, P.C., and Baril, E., Proc. natl Acad. Sci. USA 78 (1981) 838-842.
- 312 Rapaport, E., Zamecnik, P.C., and Baril, E., J. biol. Chem. 256 (1981) 12148-12151.
- 313 Grummt, F., and Hübscher, U., (1982) unpublished results.
- 314 Garison, P.N., Culver, C.A., and Bernes, L.A., Fedn Proc. 40 (1981) 1905.
- 315 Sasakawa, L., Uno, Y., and Yoshikawa, M., Molec. gen. Genet. 182 (1981) 19-24.
- 316 Wickner, W., Scheckman, R., Geider, K., and Kornberg, A., Proc. natl Acad. Sci. USA 70 (1973) 1764-1767.
- 317 Scheckman, R., Weiner, A., and Kornberg, A., Science 186 (1974) 987-993.
- 318 Eisenberg, S., Scott, J.F., and Kornberg, A., Proc. natl Acad. Sci. USA 73 (1976) 3151-3155.
- 319 McHenry, C.S., and Kornberg, A., in: The Enzymes, vol. 14, pp. 39-50. Ed. P. Boyer. Academic Press, New York 1982.
- 320 Albert, W., Grummt, F., Hübscher, U., and Wilson, S.H., Nucl. Acids Res. 10 (1982) 935-946.
- 321 Grummt, F., Wärtl, G., Jantzen, H.M., Hamprecht, K., Hübscher, U., and Kuenzle, C.C., in: Regulation of macromolecular synthesis by low molecular mediators, pp. 209-221. Eds G. Koch and D. Richter. Academic Press, New York 1979.
- 322 Johanson, K.O., and Mc Henry, C.S., in: Structure and DNA-protein interactions of replication origins, pp. 425-436. Eds D.S. Ray and C.F. Fox. Academic Press, New York 1981.
- 323 Di Francesco, R.A., Hardy, M.A., and Bessman, M.J., Fedn Proc. 40 (1981) 1763.
- 324 Burgers, P.M.J., Low, R.L., Kobori, J., Fuller, R., Kaguni, J., Stayton, M.M., Flynn, J., Bertsch, L.R., Taylor, K., and Kornberg, A., in: Structure and DNA-protein interactions, pp. 409-423. Eds D.S. Ray and C.F. Fox. Academic Press, New York 1981.
- 325 Meyer, R.R., Shlomai, J., Kobori, J., Bates, D.L., Rowen, L., Mc Macken, R., Ueda, K., and Kornberg, A., Cold Spring Harb. Symp. quant. Biol. 43 (1979) 289-293.
- 326 de Waard, A., Paul, A.V., and Lehman, I.R., Proc. natl Acad. Sci. USA 54 (1965) 1241-1248.
- 327 Alberts, B.M., and Frey, L., Nature 227 (1970) 1313-1318.
- 328 Sinha, N.K., Morris, C.F., and Alberts, B.M., J. biol. Chem. 255 (1980) 4290-4303.
- 329 Hibner, U., and Alberts, B.M., Nature 285 (1979) 300-305.
- 330 Piperno, J.R., and Alberts, B.M., J. biol. Chem. 253 (1978) 5174-5179.
- 331 Huang, C.-C., Hearst, J.E., and Alberts, B.M., J. biol. Chem. 256 (1981) 4087-4094.

- 332 Morris, C.F., Hama-Inaba, H., Mace, D., Sinha, N.K., and Alberts, B.M., *J. biol. Chem.* 254 (1979) 6787-6796.
- 333 Hübscher, U., (1980) unpublished results.
- 334 McHenry, C., in: *Mechanistic Studies of DNA Replication and Genetic Recombination*, pp.569-577, Ed. B.M. Alberts, Academic Press, New York 1980.
- 335 Burgers, P.M.J., and Kornberg, A., *J. biol. Chem.* 257 (1982) 11468-11473.
- 336 Wickner, S., and Hurwitz, J., *Proc. natl Acad. Sci. USA* 73 (1976) 1053-1057.
- 337 Weiner, J.H., Bertsch, L.R., and Kornberg, A., *J. biol. Chem.* 250 (1975) 1972-1980.
- 338 Spanos, A., and Hübscher, U., in: *Methods in Enzymology*, vol.91, pp.263-277. Eds S.P. Colowich and N.O. Kaplan. Academic Press, New York 1982.
- 339 Hesslewood, I.P., Holmes, A.M., Wakeling, W.F., and Johnston, I.R., *Eur. J. Biochem.* 84 (1978) 123-131.
- 340 Matsukage, A., Sivarajan, M., and Wilson, S.H., *Biochemistry* 15 (1976) 5305-5314.
- 341 Randerath, K., Janeway, C.M., Stephensen, M.L., and Zamecnik, P.C., *Biochem. biophys. Res. Commun.* 24 (1966) 98-105.
- 342 Herrick, G., and Alberts, B.M., *J. biol. Chem.* 251 (1976) 2124-2132.
- 343 Herrick, G., and Alberts, B.M., *J. biol. Chem.* 251 (1976) 2133-2141.
- 344 Herrick, G., Delius, H., and Alberts, B.M., *J. biol. Chem.* 251 (1976) 2142-2146.
- 345 Duguet, M., Soussi, T., Rossignol, J.M., Méchali, M., and de Recondo, A.-M., *FEBS Lett.* 79 (1977) 160-164.
- 346 Richter, A., Knippers, R., and Otto, B., *FEBS Lett.* 91 (1978) 293-296.
- 347 Méchali, M., and de Recondo, A.-M., *Biochem. biophys. Res. Commun.* 82 (1978) 255-264.
- 348 Duguet, M., and de Recondo, A.-M., *J. biol. Chem.* 253 (1978) 1660-1666.
- 349 Riva, S., Clivio, A., Valentini, O., and Cobianchi, F., *Biochem. biophys. Res. Commun.* 96 (1980) 1053-1062.
- 350 Dause, J.M., Egly, J.M., and Kempf, J., *FEBS Lett.* 124 (1981) 84-88.
- 351 Cobianchi, F., Riva, S., Mastromei, G., Spadari, S., Pedrali Noy, G., and Falaschi, A., *Cold Spring Harb. Symp. quant. Biol.* 43 (1979) 639-647.
- 352 Morioka, K., and Terayama, H., *Biochem. biophys. Res. Commun.* 61 (1974) 568-575.
- 353 Novak, B., and Baril, E., *Nucl. Acids Res.* 5 (1978) 221-239.
- 354 Kalf, G.F., Mettrione, R.M., and Koszalka, T.R., *Biochem. biophys. Res. Commun.* 100 (1981) 566-575.
- 355 Gauz, P.R., and Pearlman, R.E., *Eur. J. Biochem.* 113 (1980) 159-173.
- 356 Murakami-Murofushi, K., and Mano, Y., *Biochim. biophys. Acta* 475 (1977) 254-266.
- 357 Bhattacharya, P., Simet, I., and Basu, S., *Proc. natl Acad. Sci. USA* 78 (1981) 2683-2687.
- 358 Blue, W.T., and Weissbach, A., *Biochem. biophys. Res. Commun.* 84 (1978) 603-610.
- 359 Mosbaugh, D.W., Stalker, D.M., Probst, G.S., and Meyer, R.R., *Biochemistry* 16 (1976) 1512-1518.
- 360 Matsukage, A., Tanabe, K., Yamaguchi, M., Taguchi, Y., Nishizawa, M., Takahashi, T., and Takahashi, T., *Biochim. biophys. Acta* 655 (1981) 269-277.
- 361 Tomizawa, J., and Selzer, G., *A. Rev. Biochem.* 48 (1979) 999-1034.
- 362 Shlomai, J., and Kornberg, A., *Proc. natl Acad. Sci. USA* 77 (1980) 799-803.
- 363 Eisenberg, S., Griffith, J., and Kornberg, A., *Proc. natl Acad. Sci. USA* 74 (1977) 3198-3203.
- 364 Mansfeld, A.D.M., Langeveld, S.A., Baas, P.D., Jansz, H.S., van der Marel, G.A., Veeneman, G.H., and van Boom, J.H., *Nature* 288 (1980) 561-566.
- 365 Mc Macken, R., Ueda, K., and Kornberg, A., *Proc. natl Acad. Sci. USA* 74 (1977) 4190-4194.
- 366 Rowen, L., and Kornberg, A., *J. biol. Chem.* 253 (1978) 758-764.
- 367 Nossal, N.G., *J. biol. Chem.* 249 (1974) 5668-5676.
- 368 Nossal, N.G., and Peterlin, B.M., *J. biol. Chem.* 254 (1979) 6032-6037.
- 369 Liu, L.F., Liu, C.C., and Alberts, B.M., *Nature* 281 (1979) 456-461.
- 370 Eisenberg, S., Scott, J.F., and Kornberg, A., *Cold Spring Harb. Symp. quant. Biol.* 43 (1979) 295-302.
- 371 Scott, J.F., Eisenberg, S., Bertsch, L.R., and Kornberg, A., *Proc. natl Acad. Sci. USA* 74 (1977) 193-197.
- 372 Arai, N., Arai, K.-I., and Kornberg, A., *J. biol. Chem.* 256 (1981) 5287-5293.
- 373 Arai, N., and Kornberg, A., *J. biol. Chem.* 256 (1981) 5294-5298.
- 374 Abdel-Monem, M., and Hoffmann-Berling, H., *Trends biochem. Sci.* 5 (1980) 128-130.
- 375 Gellert, M., Mizuuchi, K., O'Dea, M.H., and Nash, H., *Proc. natl Acad. Sci. USA* 73 (1976) 3872-3876.
- 376 Champoux, J., *A. Rev. Biochem.* 47 (1978) 449-479.
- 377 Challberg, M., Desiderio, S.V., and Kelly, T., *Proc. natl Acad. Sci. USA* 77 (1980) 5105-5109.
- 378 Enomoto, T., Lichy, J.H., Ikeda, J.-E., and Hurwitz, J., *Proc. natl Acad. Sci. USA* 78 (1981) 6779-6783.
- 379 Hoch, S.O., and Mc Vey, E., *J. biol. Chem.* 252 (1977) 1881-1887.
- 380 Otto, B., *FEBS Lett.* 73 (1977) 17-24.
- 381 Reinhard, P., and Müller, B., *FEBS Lett.* 113 (1980) 61-64.
- 382 Falaschi, A., Cobianchi, F., and Riva, S., *Trends biochem. Sci.* 5 (1980) 154-157.
- 383 Otto, B., *FEBS Lett.* 79 (1977) 175-178.
- 384 Miller, K.G., Liu, L.R., and Englund, P.T., *J. biol. Chem.* 256 (1981) 9334-9339.
- 385 Eliasson, P., and Reichard, P., *Nature* 272 (1978) 184-186.
- 386 Alberts, B.M., Barry, J., Bittner, M., Davies, M., Hama-Inaba, H., Liu, C.-C., Mace, D., Moran, L., Morris, C.F., Piperno, J., and Sinha, N.K., in: *Nucleic Acid-Protein Recognition*, pp.31-63. Ed. H.J. Vogel. Academic Press, New York 1977.
- 387 Matson, S.W., Capaldo-Kimball, F.N., and Bambara, R.A., *J. biol. Chem.* 253 (1978) 7851-7856.
- 388 Mastromei, G., Eliasson, R., and Reichard, P., *J. molec. Biol.* 151 (1981) 627-643.
- 389 Bernardi, F., and Ninio, J., *Biochimie* 60 (1978) 1083-1095.
- 390 Topal, M.D., and Fresco, J.R., *Nature* 263 (1976) 285-289.
- 391 Goldberg, A.L., and Dice, J.F., *A. Rev. Biochem.* 43 (1974) 835-869.
- 392 Bessman, M.J., Muzyczka, N., Goodman, M.F., and Schnaar, R.L., *J. molec. Biol.* 88 (1974) 409-421.
- 393 Hopfield, J.J., *Proc. natl Acad. Sci. USA* 71 (1974) 4135-4139.
- 394 Ninio, J., *Biochimie* 57 (1975) 587-595.
- 395 Galas, D.J., and Branscomb, E.W., *J. molec. Biol.* 124 (1978) 653-687.
- 396 Clayton, L.V., Goodman, M.F., Branscomb, E.W., and Galas, D.J., *J. biol. Chem.* 254 (1979) 1902-1912.
- 397 Speyer, J.F., Karan, J.D., and Lemy, A.B., *Cold Spring Harb. Symp. quant. Biol.* 31 (1966) 693-697.
- 398 Mildvan, A.S., *A. Rev. Biochem.* 43 (1974) 357-399.
- 399 Das, S.K., and Fujimura, R.K., *J. Virol.* 20 (1976) 70-77.
- 400 Gillin, F.D., and Nossal, N.G., *J. biol. Chem.* 251 (1976) 5219-5224.
- 401 Gillin, F.D., and Nossal, N.G., *J. biol. Chem.* 251 (1976) 5225-5232.
- 402 Lindahl, T., *Prog. nucl. Acid Res. molec. Biol.* 22 (1979) 135-192.
- 403 Deutsch, W.H., and Linn, S., *Proc. natl Acad. Sci. USA* 76 (1979) 141-144.
- 404 Wagner, R., and Meselson, M., *Proc. natl Acad. Sci. USA* 73 (1976) 4135-4139.
- 405 Glickman, B.W., and Radman, M., *Proc. natl Acad. Sci. USA* 77 (1980) 1063-1067.
- 406 Detera, S.D., Becerra, S.P., Swack, J.H., and Wilson, S.H., *J. biol. Chem.* 256 (1981) 6933-6943.
- 407 Hall, Z.W., and Lehman, I.R., *J. molec. Biol.* 36 (1968) 321-333.
- 408 Linn, S., Kairis, M., and Holliday, R., *Proc. natl Acad. Sci. USA* 73 (1976) 2818-2822.
- 409 Salisbury, J.G., O'Connor, P.J., and Saffhill, R., *Biochim. biophys. Acta* 517 (1978) 181-185.
- 410 Krauss, S.W., and Linn, S., *Biochemistry* 19 (1980) 220-228.
- 411 Dube, D.K., Kunkel, T.A., Seal, G., and Loeb, L.A., *Biochim. biophys. Acta* 561 (1979) 369-382.
- 412 Weymouth, L.A., and Loeb, L.A., *Proc. natl Acad. Sci. USA* 75 (1978) 1924-1928.
- 413 Kunkel, T.A., and Loeb, L.A., *Science* 213 (1981) 765-767.

- 414 Sinha, N.K., and Haimes, M.D., in: *Mechanistic Studies of DNA Replication and Genetic Recombination*, pp.707-723. Eds B.M. Alberts and C.F. Fox. Academic Press, New York 1980.
- 415 Fersht, A.R., and Knill-Jones, J.W., *Proc. natl Acad. Sci. USA* 78 (1981) 4251-4255.
- 416 Kunkel, T.A., Meyer, R.R., and Loeb, L.A., *Proc. natl Acad. Sci. USA* 76 (1979) 6331-6335.
- 417 Fry, M., Shearman, C.W., Martin, G.M., and Loeb, L.A., *Biochemistry* 19 (1980) 5939-5946.
- 418 Murray, V., and Holliday, R., *J. molec. Biol.* 146 (1981) 55-76.
- 419 Fry, M., and Weisman-Shomer, P., *Biochemistry* 15 (1976) 4319-4329.
- 420 Agarwal, S.S., Tuffier, M., and Loeb, L.A., *J. Cell Physiol.* 96 (1978) 235-244.
- 421 Fry, M., Loeb, L.A., and Martin, G.M., *J. Cell Physiol.* 106 (1981) 435-444.
- 422 Murray, V., *Mech. Age. Dev.* 16 (1981) 327-343.
- 423 Davis, B.D., and Tai, D.-C., *Nature* 283 (1980) 433-438.
- 424 Burstein, Y., and Schlechter, I., *Proc. natl Acad. Sci. USA* 74 (1977) 716-720.
- 425 Roberts, J.W., Roberts, C.W., Craig, N.L., and Phizicky, E.M., *Cold Spring Harb. Symp. quant. Biol.* 43 (1979) 917-920.
- 426 Shlomai, J., Arai, K.-I., Arai, N., Kobori, J., Polder, L., Low, R., Hübscher, U., Bertsch, L.R., and Kornberg, A., in: *Mechanistic Studies of DNA Replication and Genetic Recombination*, pp.545-568. Eds B.M. Alberts and C.F. Fox. Academic Press, New York 1980.
- 427 Arai, K.-I., Arai, N., Shlomai, J., Kobori, J., Polder, L., Low, R., Hübscher, U., Bertsch, L.R., and Kornberg, A., *Prog. nucl. Acid Res. molec. Biol.* 26 (1981) 9-33.
- 428 Brutlag, D., Atkinson, M.R., Setlow, P., and Kornberg, A., *Biochem. biophys. Res. Commun.* 37 (1969) 982-989.
- 429 Klenow, H., and Henningson, I., *Proc. natl Acad. Sci. USA* 65 (1970) 168-175.
- 430 Setlow, P., Brutlag, D., and Kornberg, A., *J. biol. Chem.* 247 (1972) 224-231.
- 431 Setlow, P., and Kornberg, A., *J. biol. Chem.* 247 (1972) 232-240.
- 432 Hecht, N.B., *Nature New Biol.* 245 (1973) 199-201.
- 433 Tanabe, K., and Takahashi, T., *Biochem. biophys. Res. Commun.* 53 (1973) 295-301.
- 434 Hecht, N.B., *Biochim. biophys. Acta* 383 (1975) 388-398.
- 435 Holmes, A.M., Hesslewood, I.R., and Johnston, I.R., *Eur. J. Biochem.* 43 (1974) 487-499.
- 436 Holmes, A.M., Hesslewood, I.R., and Johnston, I.R., *Nature* 255 (1975) 420-422.
- 437 Penner, P.E., *Can. J. Biochem.* 57 (1979) 1026-1029.
- 438 Brakel, C.L., and Blumenthal, A.B., *Eur. J. Biochem.* 88 (1978) 351-362.
- 439 Pedrali Noy, G., and Weissbach, A., *Biochim. biophys. Acta* 477 (1977) 70-83.
- 440 Chang, L.M.S., *Science* 191 (1976) 1183-1185.
- 441 Chang, L.M.S., Cheriathundam, E., Mahoney, E.M., and Cerami, A., *Science* 208 (1980) 510-511.
- 442 Scovassi, A.I., Wicker, R., and Bertazzoni, U., *Eur. J. Biochem.* 100 (1979) 491-496.
- 443 Chang, L.M.S., Plevani, P., and Bollum, F.J., *Proc. natl Acad. Sci. USA* 79 (1982) 758-761.
- 444 Sala, F., Amileni, A.R., Parisi, B., and Spadari, S., *Eur. J. Biochem.* 112 (1980) 211-217.
- 445 Oguro, M., Suzuki-Hori, C., Nagano, H., Mano, Y., and Ikegami, S., *Eur. J. Biochem.* 97 (1979) 603-607.
- 446 Pedrali Noy, G., and Spadari, S., *J. Virol.* 36 (1980) 457-464.

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